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14. ABSTRACT

The U.S. Air Force Medical Service presented the fifth annual Air Force Medical Research Symposium coordinated by the Air Force Medical Support Agency's Research and Development Division (AFMSA/SGRS). The symposium was held 24-26 August 2010 at the Doubletree Hotel Washington DC – Crystal City, Arlington, VA. The symposium featured two half-days of plenary sessions, one and a half days of scientific presentations, and a poster session. It was organized into four tracks to include: Operational & Medical, Enroute Care, Force Health Protection, and Nursing. These proceedings are organized into five volumes to include one that provides a general overview and all presentation and poster abstracts; the other four each address a specific track. Volume 4 contains abstracts and presentation slides for the Force Health Protection Track.

15. SUBJECT TERMS

US Air Force, Medical Service, Medical Research, Force Health Protection

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Proceedings of the 2010 AFMS Medical Research Symposium

Volume 4. Force Health Protection Track Abstracts and Presentations

Volume 4 of 5

Proceedings of the 2010 AFMS Medical Research Symposium Volume 4. Force Health Protection Track Abstracts and Presentations

Edited by: Dr. Welford C. Roberts

Held
24-26 August 2010
at the
DoubleTree Hotel Washington DC – Crystal City
300 Army Navy Drive
Arlington, VA 22202

Table of Contents

Proceedings of the 2010 AFMS Medical Research Symposium

Introduction

The U.S. Air Force Medical Service presented the fifth annual Air Force Medical Research Symposium coordinated by the Air Force Medical Support Agency's Research and Development Division (AFMSA/SGRS). The symposium was held on 24-26 August 2010 in the Washington D.C. area at the Doubletree Hotel Washington DC – Crystal City in Arlington, VA. The symposium featured two half-days of plenary sessions, one and a half days of scientific presentations, and a poster session.

The symposium was organized into several tracks to include Operational & Medical, En-route Care, Force Health Protection, and Nursing, as follows:

- The Operational & Medical Track focused on patient care and treatment in garrison, expeditionary care during contingency operations, and enhancing performance of airman in challenging environments.
- The En-route Care Track addressed science and technology targeted at the continuum of care during transport from point of injury to definitive care to include medivac, aeromedical evacuation, critical care air transport, patient staging, and patient safety.
- The Force Health Protection Track focused on prevention of injury and illness and the early recognition or detection of emerging threats for in-garrison or deployed operations. Topics of interest include research in bio-surveillance, infectious disease, emerging threats (pandemic response), protective countermeasures, disaster response/consequence management, toxicology/health risks (e.g., particulates nanomaterials, radiation, etc.), monitoring disease trends, other areas of preventive medicine, public and environmental health relevant to the military workforce.
- The Nursing Track focused specifically on evidence based practice.

These proceedings are organized into five volumes, as follows:

- Volume 1. This volume is a general overview of the entire 2010 Air Force Medical Research Symposium and includes abstracts of all the oral presentations and posters. First presented is the symposium's opening plenary session, followed by the abstracts from the four technical tracks, and then the closing plenary session. The abstracts associated with the poster session are in the last section of these proceedings. The agenda for the overall symposium is in Appendix A, attendees are listed in Appendix B, and continuing education information is in Appendix C of this volume. Appendices D-L are copies of presentation slides from the plenary sessions.
- Volume 2. This volume contains abstracts and presentation slides for the Operational & Medical Track.
- Volume 3. This volume contains abstracts and presentation slides for the En-route Care Track.
- Volume 4. This volume contains abstracts and presentation slides for the Force Health Protection Track.
- Volume 5. This volume contains abstracts and presentation slides for the Nursing Track.

A Novel Approach to Zoonotic Population Health Monitoring: The Zoonoses Integration Project

82nd AMDS

Maj Thomas Doker

Zoonotic diseases comprise most of the pathogens that currently cause human disease and are potential bioterrorism and emerging infectious disease agents. Delays of various lengths can occur between initial diagnosis and reporting to local public health systems with traditional passive disease reporting. Animal reservoirs, vectors, and hosts create a multifaceted epidemiology. Environmental factors resulting from weather and geological events, human interactions, and habitat modifications affect the populations of animals within zoonotic disease chains of infection.

The Zoonoses Integration Project (ZIP) was designed to be a component of a fusion center that assimilated public health studies, general media sources, and other sources to generate a daily SA report. Many public health administrators do not have the time nor the expertise to gather information which provide the SA they require on a daily basis. Moreover, disease events in other countries can rapidly become global public health concerns.

ZIP provided linkage of pathogen selections to diseases and provided an effective way for listing existing subtypes. Options are recommended for selecting reservoir, vector, and host species. Daily multidisciplinary meetings were important for assessing the reliability, validity, and significance of collected data. More research is needed to determine the biosurveillance needs of decision makers and to evaluate the effectiveness of any public health action that occurs due to the receipt of timely and quality biosurveillance reports.

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A Novel Approach to Zoonotic Population Health Monitoring: The Zoonoses Integration Project

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Public Health Flight Commander
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The Zoonoses Integration Project (ZIP)

- Importance of Zoonotic Diseases
- Passive/Active Biosurveillance
- Spectrum of Zoonotic Diseases
- Need for Multidisciplinary Approach for Biosurveillance
- Fusion Center Concept
- ZIP Format/Flowchart
- Strengths/Challenges
- Recommendations

CDC

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Zoonotic Disease

World Health Organization

OIE

- Definition: any disease or infection that is naturally transmissible from vertebrate animals to humans (WHO, 2009)
- "Animals thus play an essential role in maintaining zoonotic infections in nature. Zoonoses may be bacterial, viral, or parasitic, or may involve unconventional agents" (WHO, 2009)

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Importance of Zoonotic Disease
BORDER CROSSING
AHHH.. IT FEELS GOOD TO NOT BE NEEDED FOR ONCE!
• 60% of human pathogens
• 75% of emerging diseases
• 80% of potential bioterrorism agents
• Economic losses
• Overwhelmed public health systems
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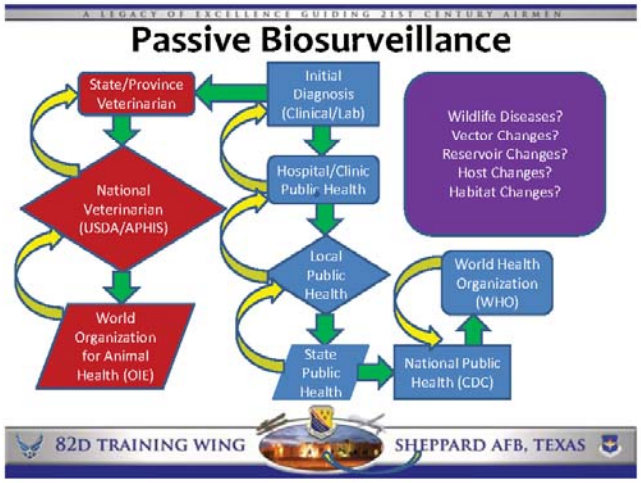
A LEGACY OF EXCELLENCE GUIDING 21ST CENTURY AIRMEN
Passive Biosurveillance
State/Province Veterinarian
Initial Diagnosis (Clinical/Lab)
Wildlife Diseases?
Vector Changes?
Reservoir Changes?
Host Changes?
Habitat Changes?
National Veterinarian (USDA/APHIS)
Hospital/Clinic Public Health
Local Public Health
World Health Organization (WHO)
World Organization for Animal Health (OIE)
State Public Health
National Public Health (CDC)
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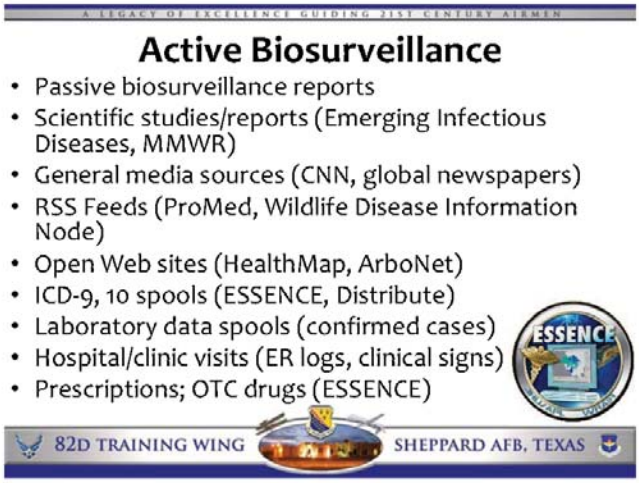
A LEGACY OF EXCELLENCE GUIDING 21ST CENTURY AIRMEN
Active Biosurveillance
• Passive biosurveillance reports
• Scientific studies/reports (Emerging Infectious Diseases, MMWR)
• General media sources (CNN, global newspapers)
• RSS Feeds (ProMed, Wildlife Disease Information Node)
• Open Web sites (HealthMap, ArboNet)
• ICD-9, 10 spools (ESSENCE, Distribute)
• Laboratory data spools (confirmed cases)
• Hospital/clinic visits (ER logs, clinical signs)
• Prescriptions; OTC drugs (ESSENCE)
ESSENCE
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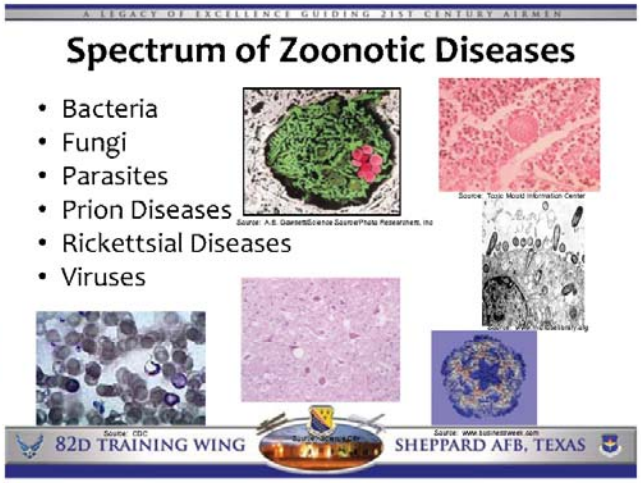
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Spectrum of Zoonotic Diseases
• Bacteria
• Fungi
• Parasites
• Prion Diseases
• Rickettsial Diseases
• Viruses
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Bacteria
Anthrax
Botulism
Brucellosis
Campylobacteriosis
Cat Scratch Disease
Escherichia coli O157:H7
Glanders
Leptospirosis
Listeriosis
Lyme Disease
Plague
Psittacosis
Q Fever
Rat Bite Fever
Salmonellosis
Streptococcosis
Tuberculosis
Tularemia
Yersiniosis
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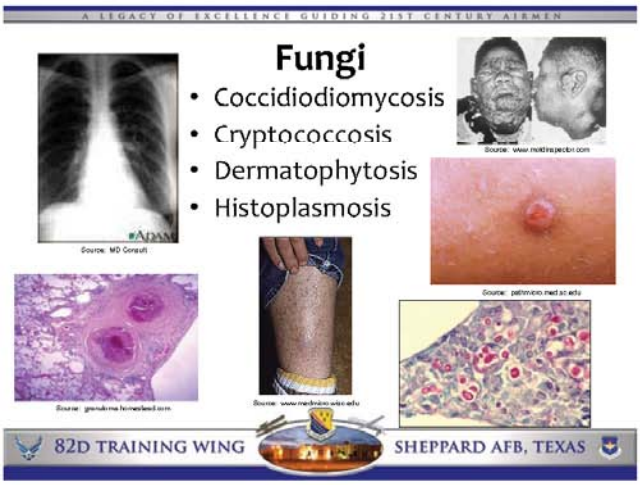
A LEGACY OF EXCELLENCE GUIDING 21ST CENTURY AIRMEN
Fungi
Coccidiodiomycosis
Cryptococcosis
Dermatophytosis
Histoplasmosis
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Parasites
African Trypanosomiasis
Babesiosis
Baylisascariasis
Chagas' Disease
Cryptosporidiosis
Cysticercosis
Echinococcosis
Hookworm Infection
Larva migrans (Cutaneous, Ocular, Visceral)
Leishmaniasis (Cutaneous, Visceral)
Sarcoptic Mange
Toxocariasis
Toxoplasmosis
Trichinosis
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Prion Diseases
Bovine Spongiform Encephalopathy
Transmissible Spongiform Encephalopathies
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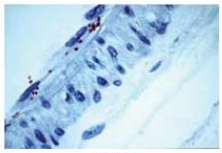
Rickettsial Diseases



Source: CDC

Source: www.vetmed.ucdavis.edu

- Boutonneuse Fever
- Rocky Mountain Spotted Fever
- Typhus Fever

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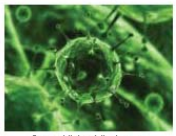
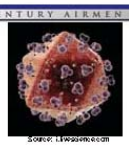
Viruses



Source: static.howstuffworks.com



Source: i.livescience.com

- Avian Influenza
- Barnah Forest Virus
- Bolivian Hemorrhagic Fever
- Bornavirus Infection
- Cache Valley Virus Disease
- Contagious Ecthyma
- Cowpox Infection
- Crimean-Congo Hemorrhagic Fever
- Dengue Fever
- Eastern Equine Encephalitis
- Ebola Virus Infection
- Hantavirus Infection
- Hendra Virus Infection
- Herpes B Infection
- Kyasanur Forest Virus
- Lassa Fever
- Lymphocytic Choriomeningitis

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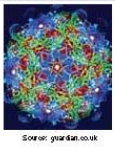
Viruses



Source: guardian.co.uk

- Marburg Fever
- Mediterranean Spotted Fever
- Monkeypox Infection
- Nipah Fever
- Omsk Hemorrhagic Fever
- Oropouche Fever
- Rabies
- Rift Valley Fever
- SARS
- Swine Influenza
- St Louis Encephalitis
- Venezuelan Equine Encephalitis
- Venezuelan Hemorrhagic Fever
- Western Equine Encephalitis
- West Nile Virus
- Yellow Fever

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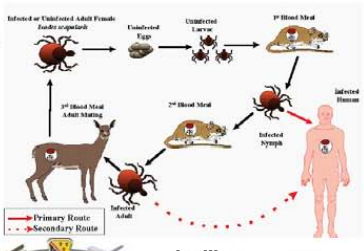
Need for Multidisciplinary Approach for Biosurveillance

- Zoonotic diseases more complex
  - Multiple animals often involved
    - Reservoirs
      - Domestic animals
      - Companion animals
      - Wildlife species
    - Vectors
    - Hosts
      - Primary
      - Secondary
      - Intermediate
      - Paratenic
      - Amplifying
      - Dead-end



Source: CDC

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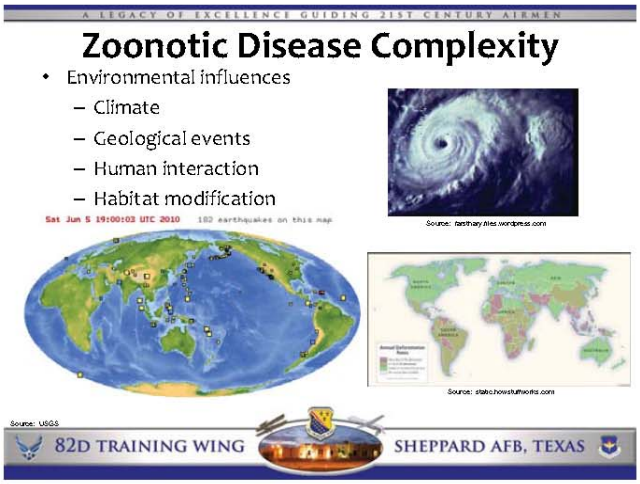
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Zoonotic Disease Complexity
• Environmental influences
– Climate
– Geological events
– Human interaction
– Habitat modification
Sat Jun 5 19:00:03 UTC 2010
Source: USGS
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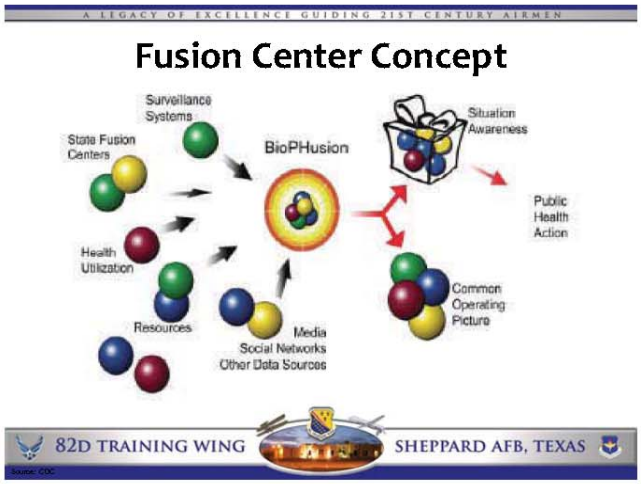
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Fusion Center Concept
Surveillance Systems
State Fusion Centers
BioPHusion
Situation Awareness
Public Health Action
Health Utilization
Resources
Media
Social Networks
Other Data Sources
Common Operating Picture
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Fusion Center Data Sources
Wildlife Disease Information Node
EMERGING INFECTIOUS DISEASES
MMWR
Morbidity and Mortality Weekly Report
twitter
• Public health studies/reports
• General media
• Active/passive biosurveillance
• Other fusion center outputs
• Social networks
The New York Times
HealthMap
ProMED-mail
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Fusion Center Subject Matter Experts
• Infectious disease docs
• Epidemiologists
• Informatics professionals
• Geographic Information Systems analysts
• Environmental health professionals
• Veterinarians
• Public health analysts
• Wildlife biologists?
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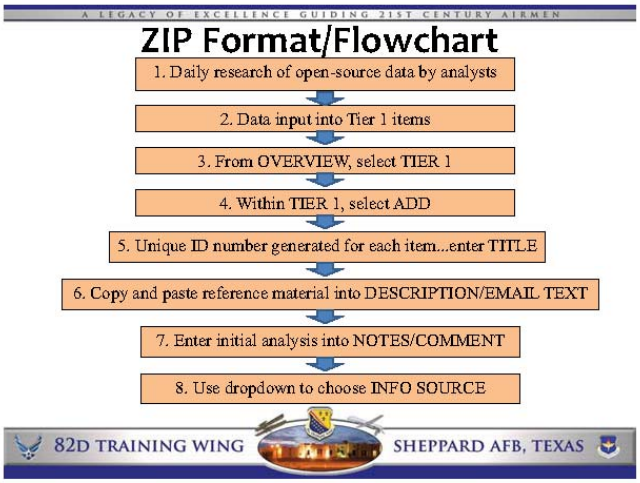
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ZIP Format/Flowchart
1. Daily research of open-source data by analysts
2. Data input into Tier 1 items
3. From OVERVIEW, select TIER 1
4. Within TIER 1, select ADD
5. Unique ID number generated for each item...enter TITLE
6. Copy and paste reference material into DESCRIPTION/EMAIL TEXT
7. Enter initial analysis into NOTES/COMMENT
8. Use dropdown to choose INFO SOURCE
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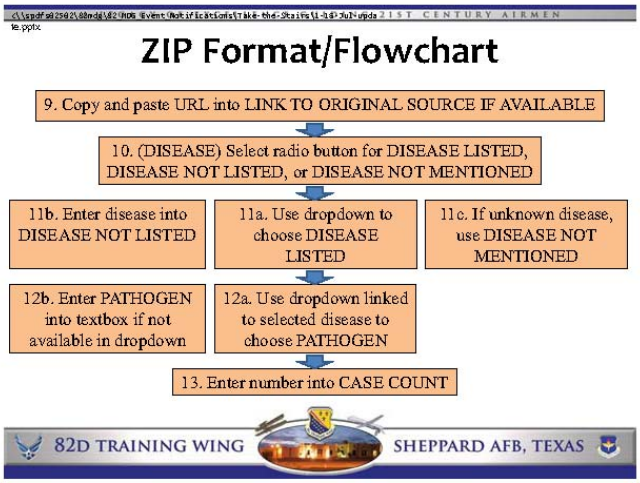
ZIP Format/Flowchart
9. Copy and paste URL into LINK TO ORIGINAL SOURCE IF AVAILABLE
10. (DISEASE) Select radio button for DISEASE LISTED, DISEASE NOT LISTED, or DISEASE NOT MENTIONED
11b. Enter disease into DISEASE NOT LISTED
11a. Use dropdown to choose DISEASE LISTED
11c. If unknown disease, use DISEASE NOT MENTIONED
12b. Enter PATHOGEN into textbox if not available in dropdown
12a. Use dropdown linked to selected disease to choose PATHOGEN
13. Enter number into CASE COUNT
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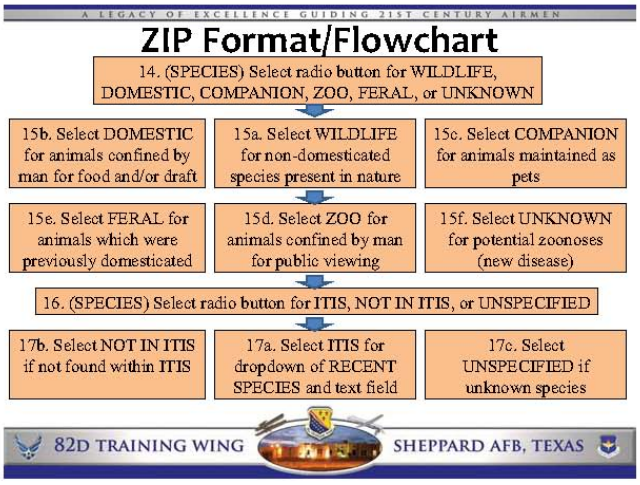
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ZIP Format/Flowchart
14. (SPECIES) Select radio button for WILDLIFE, DOMESTIC, COMPANION, ZOO, FERAL, or UNKNOWN
15b. Select DOMESTIC for animals confined by man for food and/or draft
15a. Select WILDLIFE for non-domesticated species present in nature
15c. Select COMPANION for animals maintained as pets
15e. Select FERAL for animals which were previously domesticated
15d. Select ZOO for animals confined by man for public viewing
15f. Select UNKNOWN for potential zoonoses (new disease)
16. (SPECIES) Select radio button for ITIS, NOT IN ITIS, or UNSPECIFIED
17b. Select NOT IN ITIS if not found within ITIS
17a. Select ITIS for dropdown of RECENT SPECIES and text field
17c. Select UNSPECIFIED if unknown species
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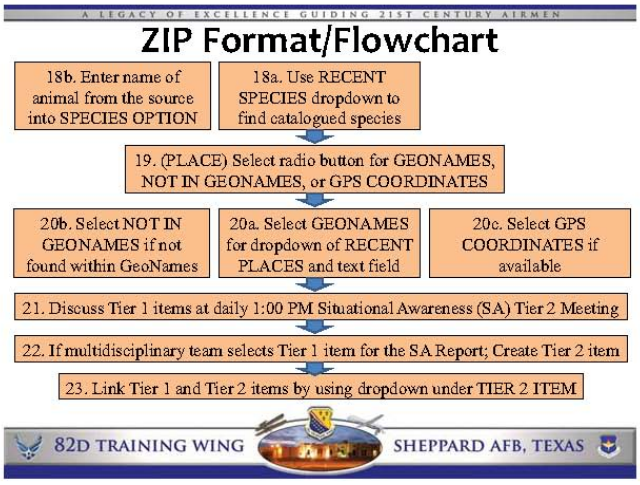
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ZIP Format/Flowchart
18b. Enter name of animal from the source into SPECIES OPTION
18a. Use RECENT SPECIES dropdown to find catalogued species
19. (PLACE) Select radio button for GEONAMES, NOT IN GEONAMES, or GPS COORDINATES
20b. Select NOT IN GEONAMES if not found within GeoNames
20a. Select GEONAMES for dropdown of RECENT PLACES and text field
20c. Select GPS COORDINATES if available
21. Discuss Tier 1 items at daily 1:00 PM Situational Awareness (SA) Tier 2 Meeting
22. If multidisciplinary team selects Tier 1 item for the SA Report; Create Tier 2 item
23. Link Tier 1 and Tier 2 items by using dropdown under TIER 2 ITEM
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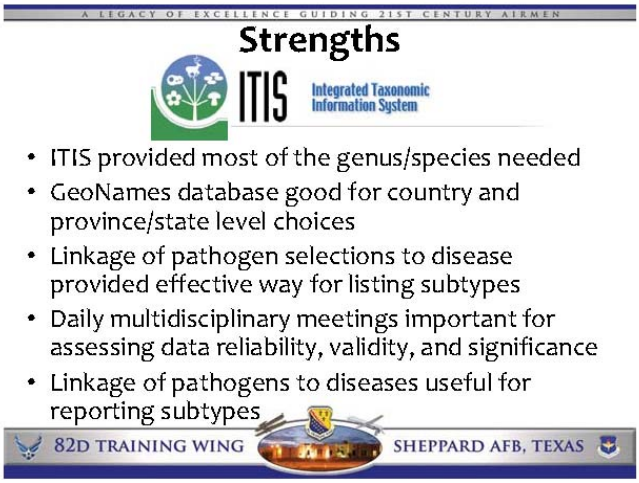
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Strengths
ITIS
Integrated Taxonomic Information System
• ITIS provided most of the genus/species needed
• GeoNames database good for country and province/state level choices
• Linkage of pathogen selections to disease provided effective way for listing subtypes
• Daily multidisciplinary meetings important for assessing data reliability, validity, and significance
• Linkage of pathogens to diseases useful for reporting subtypes
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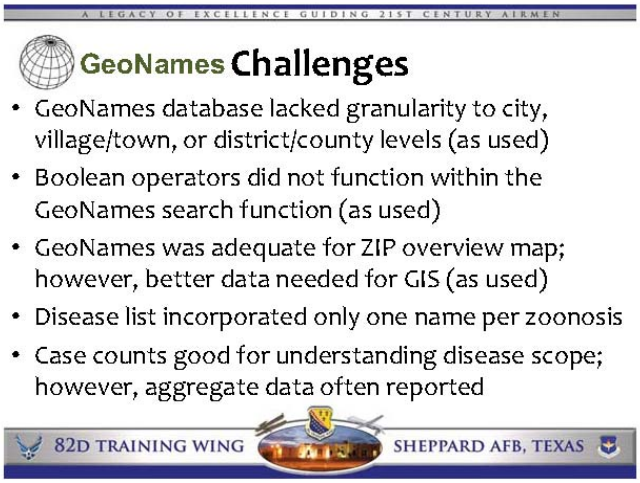
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GeoNames Challenges
• GeoNames database lacked granularity to city, village/town, or district/county levels (as used)
• Boolean operators did not function within the GeoNames search function (as used)
• GeoNames was adequate for ZIP overview map; however, better data needed for GIS (as used)
• Disease list incorporated only one name per zoonosis
• Case counts good for understanding disease scope; however, aggregate data often reported
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Recommendations
• Provide nonspecific options for pathogens
• Give options for suspect, probable, and confirmed case counts
• Incorporate active surveillance of laboratory data
• Offer animal group options (herd, flock, etc.)
• Supply estimates of disease distributions within vectors or reservoirs
• Link species list to reservoir, vector, or host options
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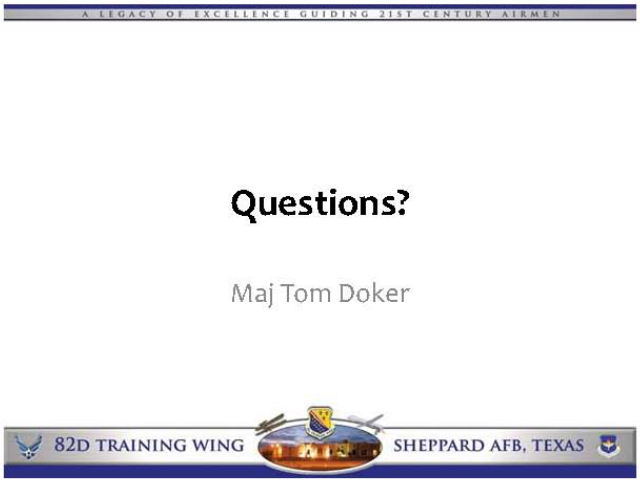
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Questions?
Maj Tom Doker
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Hydroxocobalamin and Epinephrine Each Improve Survival in a Novel Swine Model of Cyanide-Induced Cardiac Arrest: A Randomized Trial

59th Medical Wing (MDW)

Maj Vik Bebarta

INTRODUCTION: Hydroxocobalamin (HOCB) is a cyanide (CN) antidote, but it has not been studied in CN-induced cardiac arrest. In addition, a clinically relevant model for drug or chemical induced cardiac arrest has not been described. HYPOTHESIS: Our primary hypothesis was that HOCB will improve survival compared to controls in a CN-induced cardiac arrest swine model. METHODS: 45 swine were intubated and instrumented and then cyanide was infused until cardiac arrest. Animals were randomly assigned to HOCB, EPI, or saline bolus. CPR was performed with a chest compression device. Vasopressor infusion (epinephrine) was used after ROSC for SBP < 90 mm Hg. RESULTS: At 2 and 4 min after arrest, coronary perfusion pressures were greater than 15 mm Hg in treatment groups. All (15) animals in the control group, 4/15 in HOCB group, and 4/15 in EPI group died ($p < 0.001$). ROSC at 5 min and 10 min were similar in treatment groups ($p > 0.9$). Vasopressor infusion after ROSC was required for hypotension in 2/11 HOCB animals and in 11/11 EPI animals ($p < 0.001$). At 60 min, serum lactate (4.9 vs. 12.1, $p<0.0001$) and pH (7.34 vs. 7.153 $p<0.0001$) improved in the HOCB group. Serial serum CN levels in the HOCB group were lower after arrest until study end ($p<0.004$). CONCLUSIONS: HOCB and EPI both improved survival compared to controls in this swine model of cyanide induced cardiac arrest. HOCOB improved blood pressure, pH, lactate, and cyanide levels, and reduced epinephrine infusion use compared to the EPI group.

8/9/2010

Antidotal treatment for acute cyanide toxicity in a clinically relevant model

Vik Bebarta MD
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Wilford Hall Medical Center

AFMS Conference – 2010 Aug

Disclosure

- My opinions and comments do not reflect the official policy or position of the
  - Department of the Air Force
  - Department of Defense
  - US Government
- Funding - USAF Office of the Surgeon General
- No other financial disclosures
  - No industry support

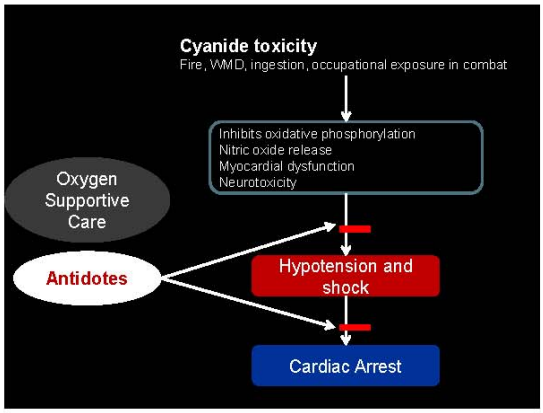
Outline

- Background
- 2 studies – cyanide induced shock
- 1 study – cyanide induced cardiac arrest

Background

- Hypotension or cardiac arrest
  - > 50% of cyanide exposed patients[1]
- IOM, NIH, CDC, and USAF agree
  - "more study is needed" for antidotal treatment
- An antidote that is effective early may be better
  - Industry sites, ambulance, ships, disaster

[1]Fortin, *Clin Toxicol*, 2006



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Hydroxocobalamin

Characteristics	HOCOB	Sodium nitrite
Few serious adverse effects	✓	
Simplicity of use	✓	
Number of drugs used	✓	
Cost		✓
Efficacy	?	?

Hydroxo vs. the Cyanide Antidote Kit in cyanide induced shock

Project title

Hydroxocobalamin and sodium thiosulfate versus sodium nitrite and sodium thiosulfate for acute cyanide toxicity treatment in a swine (*Sus Scrofa*) model – a randomized trial

Background – antidotal studies

- Older comparative studies – limited[1,2,3]
- Recent studies – limited[4,5]
- No clinically relevant large animal comparative study with cardiotoxicity has been published[6]

[1]Friedberg DK, *Arch Toxicol*, 1975 [2]Krapez, *Br J Anes*, 1981 [3]Hobel, *Arch Tox*, 1980
[4]Nick J, *Mil Med*, 2000 [5]Borron S, *Clin Toxicol*, 2006 [6]Kerns, *Ann Emerg Med*, 2007

Study Objective

- To compare the return to baseline of mean arterial pressure between 2 groups of swine toxic from acute cyanide treated with HOCOB + ST or sodium nitrite + ST

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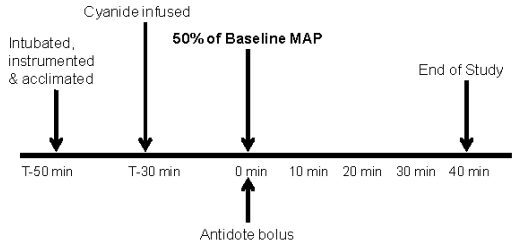
Methods

- Randomized comparative study
- Approved by local IACUC



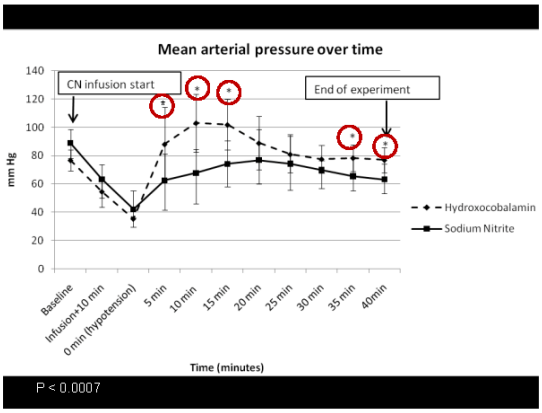
Key outcome measurements

- Primary
  - Change in mean MAP after antidote until 40 min
- Secondary
  - Change in HR, CO, mixed venous sat over time
  - Biochemical markers – pH, lactate, bicarbonate, cyanide levels

Results

- 21 of 24 animals survived to study end
- Deaths
  - 1 animal in hydroxocobalamin + ST group
  - 2 animals in the sodium nitrite + ST group

Baseline Characteristics	Hydroxocobalamin + ST group	Sodium nitrite + ST group
Weight, kg	41.6±3	42.7±5
HR, beats/min	101±15	100±14
MAP, mm Hg	78±8	87±10
CO, L/min	5.8±1.1	5.7±0.8
SVO2, %	77±9	78±7
pH	7.42±0.03	7.44±0.03
Hemoglobin, g/dL	8.05±1.0	8.5±0.6
Lactate	1.2±0.8	1.1±0.4



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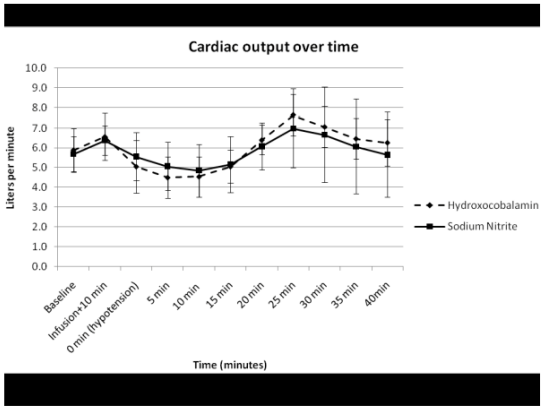
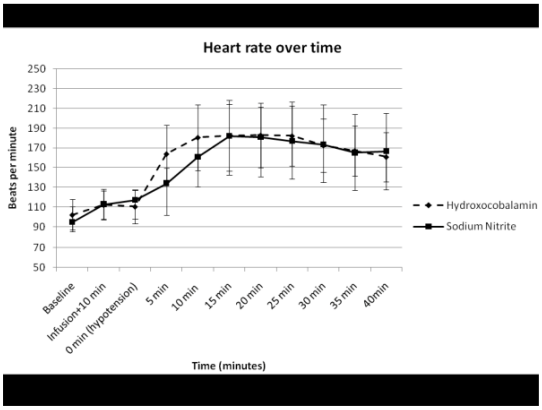
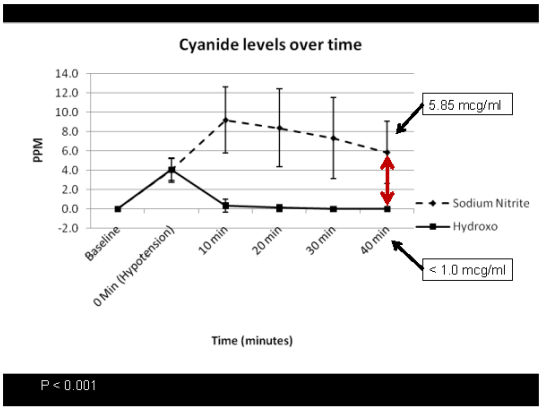




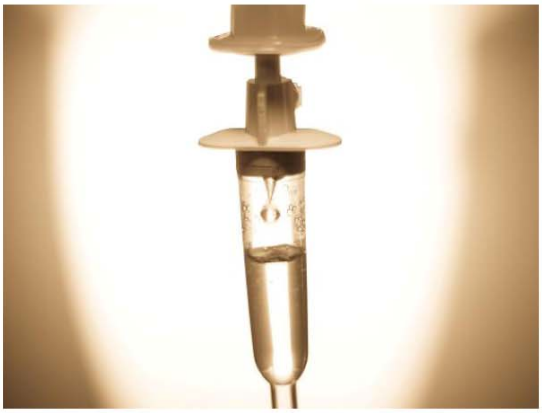
Results

- Similar mean biochemical markers at 40 min
  - Lactate, base excess, and pH
- Methemoglobin at 40 minutes
  - Sodium nitrite – 8.5%
- Hydroxocobalamin levels
  - Peaked at 10 min, with 50% reduction at 30 min



Conclusion

- In our acute CN toxic hypotensive swine model
- Hydroxocobalamin + sodium thiosulfate
  - Led to faster and sustained return to baseline MAP as compared to SN+ST
  - It significantly reduced cyanide levels
- Given HOCOB's lack of serious adverse effects
  - It may be best choice for acute cyanide toxicity



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The Follow-on study

- Would sodium thiosulfate work alone?
- Will adding ST to hydroxocobalamin improve outcome?

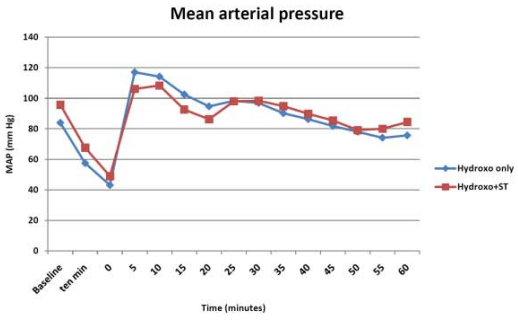
Hydroxocobalamin vs Sodium thiosulfate

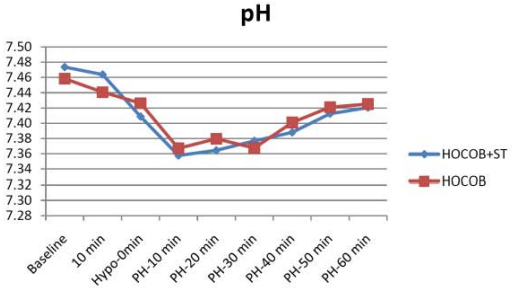
Experiment

- Same model – Extended to 60 min
- 3 Arms – Hydroxo, Sodium thiosulfate, Both
- Added
  - Nitrotyrosine – measure of nitric oxide
  - PA catheter with SVO2 AND right ventricular function

Deaths

- All 12 in sodium thiosulfate group
- 1/12 in hydroxocobalamin only group
- 2/12 in hydroxo + ST group





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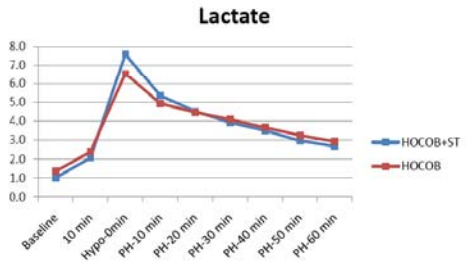


Conclusion

- In our acute CN toxic hypotensive swine model
- Sodium thiosulfate is not effective alone for severe cyanide toxicity
- Hydroxo and Hydroxo+sodium thiosulfate
  - Led to similar return to baseline MAP and deaths
  - Both reduced cyanide levels
- Given Hydroxo's lack of serious adverse effects
  - Hydroxo alone may be best choice for acute cyanide toxicity

Cyanide induced cardiac arrest study

Intro and hypothesis

- Hydroxo not studied in CN-induced cardiac arrest
  - No clinically relevant model for drug or chemical induced cardiac arrest
- 40% of cyanide cases are in cardiac arrest
- AHA – cardiac arrest is different physiology and pharmacokinetics than shock

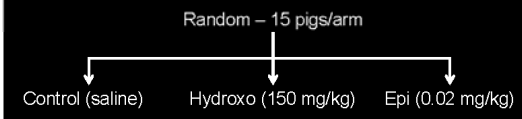
American Heart Association
Learn and Live

Hypothesis

- **Hypothesis** – Hydroxo will improve survival compared to controls in a cyanide induced cardiac arrest swine model
- Primary outcome – survival
- Secondary outcomes
  - Improvement of blood pressure, lactate, and coronary perfusion pressure with HOCB compared to epinephrine (EPI)

Methods

- 45 swine (38-42 kg)
  - Intubated, anesthetized, and instrumented
  - Continuous arterial and right atrial pressure
- Cyanide infusion until cardiac arrest



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Methods

- CPR performed with a chest compression device

- Monitored for 60 min after cardiac arrest.
- Epi infusion was used in all arms for hypotension after ROSC

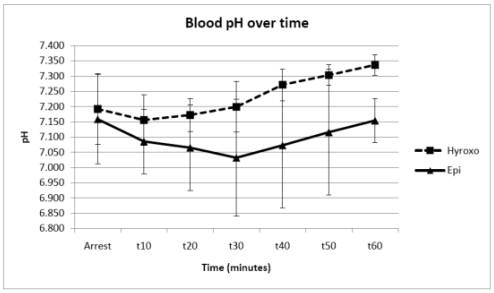
Table 1 Baseline Data

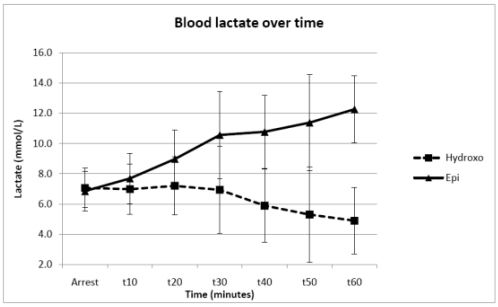
Characteristics	Control	Epinephrine	Hydroxocobalamin
Weight, kg	40±2.6	43±2.1	43±2.3
Heart rate, beats/min	89±9	89±17	90±9
Systolic blood pressure, mm Hg	100±11	111±13	115±14
Mean arterial pressure, mm Hg	79±10	89±12	94±13
pH	7.42±0.05	7.40±0.07	7.46±0.04
Hemoglobin, g/dL	9±0.6	8.3±1.0	8.4±1.0
Lactate	1.0±0.8	1.1±0.6	1.1±0.5

g/dL, grams per deciliter; **Kg**, kilograms; **min**, minute **mm**, millimeters; **ST**, sodium thiosulfate
Data presented as means ± standard deviation

Mortality

- Control – No animals (0/15) lived
- Hydroxocobalamin – 73%(11/15) animals lived
- Epinephrine – 73% (11/15) lived
- Both treatments improved survival over control ($p < 0.001$)





Summary

- Hydroxocobalamin and epinephrine each improved survival compared to controls in our model of cyanide induced cardiac arrest
- Hydroxocobalamin
  - Improved blood pressure, pH, lactate, and cyanide levels, and reduced the use of epinephrine infusion compared to the epinephrine group

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Questions
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Cold Injury in Military Population: Current Trends and Comparison to Past Conflicts with Current Research

59th 81 MSGS/SGCQ

Capt Andrew Hall

OBJECTIVE: The war in Afghanistan represents the first large-scale conflict involving military troops in a cold, mountainous climate since the Korean War. An analysis was conducted to identify the extent of cold weather injuries, especially frostbite, in the deployed military population. DESIGN: A retrospective analysis of military databases was conducted with tabulation of all cases of cold weather injuries in Operations Enduring Freedom and Iraqi Freedom. Casualties reviewed occurred between 2001 and 2009. RESULTS: A total of 19 cases of cold weather injury were identified in the Afghanistan conflict. 2 cases of frostbite were identified with only one likely requiring surgical intervention. No cases were identified in Iraq. CONCLUSIONS: The 19 cold weather injuries represents a dramatic decrease from the 6300 cases of cold weather injury seen in the last major cold weather conflict, the Korean War. This is due to the shorter and weather dependent engagements, cold weather education, and improved equipment of US and allied personnel. Discussion of research into angiogenesis using omental lipids for the treatment of frostbite and wound healing will also be discussed.

Cold Injury in Military Population: Current Trends and Comparison to Past Conflicts with Current Research

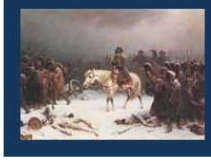
Capt. Andrew B. Hall, USAF MC
81st MSGS/SGCQ

Overview

- Background and History
- Comparison between past and present
- Discussion
- Current Research

Cold Injury in Military

- Cold has been plaguing military forces through recorded history
  - Hannibal- Crossing the Alps
    - (Approximately 50% casualties)
  - Washington- Valley Forge
    - (Approximately 17% casualties)
  - Napoleon- Retreat from Russia
    - (Approximately 80% casualties)



Cold Injury in Military

- Modern Warfare with similar casualties
  - World War I – 2,000
  - World War II – 91,000
  - Korean War – 6,300 (5,000 in 1950-1951)
- Korean War was first war where epidemiology and treatment for frostbite thoroughly investigated
- Afghanistan represents our first large scale conflict in a cold weather theater since the Korean War

Methods

- Department of Defense Registries Searched via ICD 9 Code, Name of Diagnosis and Abbreviated Injury Scale Code (AIS)
  - Trans Com Regulating and Command and Control Evacuation System (TRAC²ES)
  - Joint Theater Trauma Registry (JTTR)
  - Defense Medical Epidemiology Database (DMED)
  - Armed Forces Health Longitudinal Technology Application (AHLTA)
- All cases of non-environmental causes, if identified, were removed (i.e. burn victims in summer)

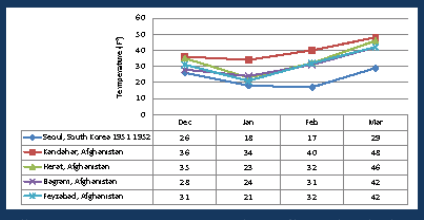
Results

- Total Reported Cases: 19
  - Frostbite: 2
  - Hypothermia: 17

Age, Operation, Month	Cold Weather Injury	Mechanism of Injury (if Known)
25, OEF, January	Hypothermia	Caught in River
21, OEF, January	Hypothermia	Caught in River
21, OEF, January	Hypothermia	Caught in River
24, OEF, March	Hypothermia	12 Hours in snow in defensive Position
26, OEF, February	Hypothermia	
21, OEF, January	Frostbite	
36, OEF, February	Hypothermia	
20, OEF, February	Frostbite	Patrol for 6 hours
19, OEF, January	Hypothermia	Caught in River
36, OEF, January	Hypothermia	Caught in River
26, OEF, January	Hypothermia	Caught in River
36, OEF, October	Hypothermia	Submersion in Water
40, OEF, January	Hypothermia	Caught in River
39, OEF, April	Hypothermia	
25, OEF, January	Hypothermia	Caught in River
32, OEF, July	Hypothermia	
24, OEF, July	Hypothermia	
41, OEF, January	Hypothermia	Caught in River
21, OEF, February	Hypothermia	

Comparison: Basic Temperature

- Temperatures in Afghanistan are warmer then then Korea
  - Changes in elevation can have a pronounced effect on temperature



	Dec	Jan	Feb	Mar
Seoul, South Korea 1951 1952	26	18	17	29
Kandahar, Afghanistan	36	34	40	48
Herat, Afghanistan	35	23	32	46
Bagram, Afghanistan	28	24	31	42
Feyzabad, Afghanistan	31	21	32	42

Table 2: Mean winter minimum temperatures at various geographic sites in Afghanistan and at Seoul, South Korea, during the winter of 1951 1952. Mean temperatures in Feyzabad, Afghanistan, were averaged from 2006 to 2008; in Bagram from 2002 to 2008; in Herat from 2007 to 2008; and in Kandahar from 2003 2008.

Comparison: Basic Temperature

- Real world frostbite depends on duration of tissue exposure to cold
  - Intercellular ice formation at -2°C

	Jan	Feb	Mar	Apr
Kandahar, Afghanistan	14	18	27	21
Herat, Afghanistan	4	1	16	9
Bagram, Afghanistan	6	7	21	9
Feyzabad, Afghanistan	2	3	19	14

Table 3: Minimum temperatures of various geographic sites in Afghanistan. Period of record for minimum temperatures in Feyzabad is from 1973 to 2009; in Bagram, from 2001 to 2008; in Herat, from 1973 to 2009; and in Kandahar, from 1973 to 2009.

Comparison: Mobility

- Mobility increased greatly in Afghanistan conflict
  - Mobility increased blood flow and heat generation
  - Uncommon prolonged engagements in Afghanistan
    - Short engagements and preplanned retreat when ground/air units arrive
    - Winter weather impedes travel
  - Ad Hoc Defenses with persistent engagement in Korean Conflict
    - 75% of 4th degree frostbite in conditions of greater then 7 hours of immobility

Comparison: Mobility

- Notable decrease in numbers of winter casualties reflect difficulty of winter travel

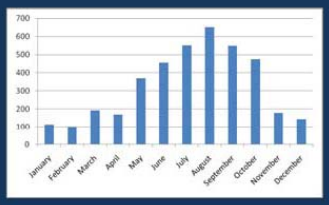


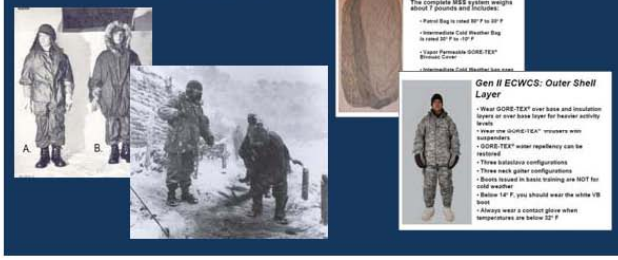
Table 4: Total number of Army casualties by month over course of Operation Enduring Freedom. Time frame includes December 4, 2001 to October 10, 2009. Source: Center for Army Lessons Learned

Comparison: Education

- Education and training is significantly improved
  - In Korean conflict, many soldiers were ill trained for prolonged winter engagements
  - Modern training facilities prepare deployed personnel for winter operations
    - USMC Mountain Warfare Training Center
    - United States Army Alaska Northern Warfare Training Center
  - Acclimation to environment is unlikely however to play a significant role

Comparison: Equipment

- Winter of 1950-1951, winter gear was not designed or distributed for the experienced winter conditions



Discussion

- JTTR database may not reflect secondary diagnoses in larger traumas
- 1st and 2nd degree frostbite and minor hypothermia treated in theater without reaching a major medical center
- Details of injury, location, and circumstances missing

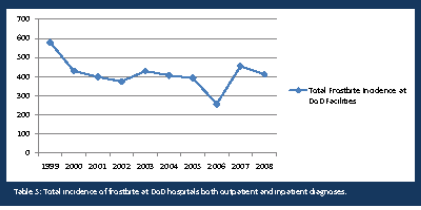


Table 1: Total incidence of frostbite at DoD hospitals both outpatient and inpatient diagnoses.

Conclusions

- Dramatic reduction in numbers of diagnosed cold injuries
- Reduction due to multiple changes since last cold battlefield
  - Better Mobility
  - Better Training
  - Better Equipment

Conclusions

- While allied forces have currently had low numbers of casualties, surgeons still must be prepared for the civilian population


Ninety Afghan frostbite victims have amputations

(Reuters) - Doctors said on Sunday they have amputated the fingers or toes of more than 90 frostbite victims in western Afghanistan as one of the worst winters in living memory engulfs the country.

Pathology and Treatment of Frostbite

- Pathology: Progressive vascular ischemia and direct cellular damage
- Treatment:
  - Don't operate
  - Rapid rewarming in 40°-42° C water for 15-30 minutes
  - Anticoagulation with Heparin, Aspirin, tPA shown experimentally to increase tissue survival
  - Hyperbaric oxygen shown to anecdotally improve tissue survival
  - Surgery in 1-3 months if dead tissue remains
- Current Research: omental angiogenic factors injected to injured site to improve tissue survival

Current Research: Angiogenesis in Frostbite

Angiogenesis in Frostbite

- Omentum facilitates angiogenesis after abdominal injury
- Refined omental lipids have been shown to be angiogenic and improve tissue survival in previous research
  - Takada T, et al. Effect of omental lipid fraction on enhancement of skin flap survival. Ann Plast Surg 1998;41:70-74
  - Nottebaert M, et al. Omental angiogenic lipid fraction and bone repair. An experimental study in the rat. J Orthop Res 1989;7(2):157-169
  - Goldsmith H, et al. Lipid angiogenic factor from omentum. JAMA 1984;252:2034-2036

Angiogenesis in Frostbite

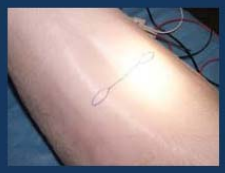
- Two main objectives

Angiogenesis in Frostbite

- Objective #1
  - Verify angiogenic potential of rabbit derived omental lipid fraction
  - Using Matrigel angiogenic assay
- Objective #2
  - Determine effect of injected omental lipids at site of frostbite.

Angiogenesis in other conditions

- Radiation and normal injury
- Improved biologic mesh incorporation



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Questions?

The Association between Stress and Physical Fitness Testing in the 2005 Department of Defense Population Survey

United States Air Force School of Aerospace Medicine (USAFSAM)

Lt Col/Dr. Valerie Johnson

OBJECTIVE: The purpose of this study was to examine the association of perceived stress and passing the fitness test in a cohort of Department of Defense active duty members. Reports of this association have been suggested in numerous articles. METHODS: The 2005 DoD Survey of Health Related Behaviors Among Active Duty Military Personnel was used to examine the association between the participants' perceived levels of stress from family and/or work related sources and the respondents' last required fitness test taking into account potential confounder of the association. Measures of association were obtained from logistic regression models. RESULTS: Participants who experienced "some" or "a lot" of stress either from work sources (OR 0.69, 95% CI: 0.58-0.87) or from personal/family sources (OR 0.70, 95% CI: 0.57-0.86) were less likely to pass the fitness test when compared to their counterparts who experienced "none" or "a little" stress. Additionally, those who reported "some" or "a lot" of stress either from work sources (OR 0.54, 95% CI: 0.41-0.70) or from personal/family sources (OR 0.54, 95% CI: 0.44-0.67) that interfered with their military duties were also less likely to pass the fitness test. The multivariate adjustment only slightly reduced the unadjusted association. CONCLUSIONS: An association exists between perceived stress levels and outcome of fitness testing. The higher the level of stress perceived, the less likely the member will pass the fitness test. Stress-related intervention might be useful to help the military members to achieve the level of fitness needed to perform their duties.

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The Association Between Stress and Physical Fitness Testing in the 2005 Department of Defense Population Survey

24 Aug 2010

Valerie V. T. Johnson, Lt Col, USAF, MC, SFS
Resident in Occupational Medicine
USAFSAM/FEER

OVERVIEW

- Introduction
  - Background
  - Study Aim
- Methods
- Results
- Discussion
- Conclusion

BACKGROUND

- Military = Select Group of Personnel
- Preservation of physical fitness & mental health = military readiness
- Mobile/Fit and Ready Force
- DoD initiated Health Behavior Survey series (DoD Survey) in 1980
  - Monitor general health trends
  - Guide health & substance abuse programs/policies

BACKGROUND

- 1986 DoD created health promotion policy to improve & maintain military personnel readiness & quality of life
  - Physical fitness and stress management
- 2005 DoD Survey assessed attainment of health promotion goals
- Healthy People 2010

BACKGROUND

STRESS AND PHYSICAL FITNESS TESTING IN THE 2005 DoD POPULATION SURVEY: IS THERE AN ASSOCIATION?

- **STRESS: HEALTH IMPACTS**
  - **"Allostatic Load" (McEwen, 1998, 2002)**
    - Factor in expression of disease ("wear and tear")
  - **Result in behavioral patterns and physiological reactivity → deleterious effects on body**
  - **Studies have suggested that chronic or recurrent state of stress may increase the risk for health problems**

BACKGROUND

STRESS AND PHYSICAL FITNESS TESTING IN THE 2005 DoD POPULATION SURVEY: IS THERE AN ASSOCIATION?

- **STRESS: HEALTH IMPACTS**
  - **Fight or flight**
  - **Past: Protective**
  - **Present: chronic life stressors can overtax individual resulting in physical and/or mental collapse (McEwen, 1998)**

BACKGROUND

STRESS AND PHYSICAL FITNESS TESTING IN THE 2005 DoD POPULATION SURVEY: IS THERE AN ASSOCIATION?

- **STRESS: IMPACT ON PERFORMANCE**
  - **Short term = may enhance performance**
  - **Chronic exposure = negatively affects performance**
  - **Potential decrease in physical effectiveness**
  - **Catabolism = a decline in physical fitness and or performance? (Booth, 2006)**

BACKGROUND

STRESS AND PHYSICAL FITNESS TESTING IN THE 2005 DoD POPULATION SURVEY: IS THERE AN ASSOCIATION?

- **STRESS IN THE MILITARY**
  - **Approximates civilian stress**
  - **"Special Stressors"**
    - increased operational tempo
    - deployments
    - prolonged family separation
    - exposure to combat

BACKGROUND

STRESS AND PHYSICAL FITNESS TESTING IN THE 2005 DoD POPULATION SURVEY: IS THERE AN ASSOCIATION?

- PHYSICAL FITNESS: HEALTH IMPACTS
  - Significant role in health promotion and maintenance
  - Decrease the incidence of all-cause mortality and cardiovascular-related deaths
  - Lower blood pressure
  - Positively affect glucose homeostasis
  - Ameliorate hyperlipidemia

BACKGROUND

STRESS AND PHYSICAL FITNESS TESTING IN THE 2005 DoD POPULATION SURVEY: IS THERE AN ASSOCIATION?

- MILITARY PHYSICAL FITNESS: RELEVANCE
  - Physical conditioning and fitness
    - optimizes mission-specific performance
    - reduces risk of injury in the warfighter
  - Currently engaged in a large, protracted, and complex combat operations around the world

STUDY AIM

STRESS AND PHYSICAL FITNESS TESTING IN THE 2005 DoD POPULATION SURVEY: IS THERE AN ASSOCIATION?

- Examine the relationship between self-perceived stress levels and self-reported fitness levels in a sample population of Department of Defense members

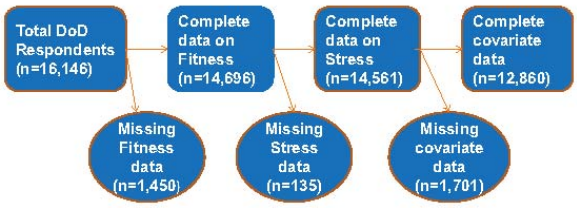
METHODS

STRESS AND PHYSICAL FITNESS TESTING IN THE 2005 DoD POPULATION SURVEY: IS THERE AN ASSOCIATION?

- Study Design: Cross-sectional Prevalence
- Data Source: 2005 DoD HRB Survey
  - April-August 2005
- Population: AD members, four branches
  - Excluded: PCS, AWOL, incarcerated, recruits and service academy cadets

METHODS: Sample Size

STRESS AND PHYSICAL FITNESS TESTING IN THE 2005 DoD POPULATION SURVEY: IS THERE AN ASSOCIATION?



INDEPENDENT VARIABLES

STRESS AND PHYSICAL FITNESS TESTING IN THE 2005 DoD POPULATION SURVEY: IS THERE AN ASSOCIATION?

- Based on four stress-related questions

"During the past 12 months,"—

- "how much stress did you experience at work or while carrying out your military duties?" (EDQ88)
- "how much stress did you experience in your family life or in a relationship with your spouse, live-in fiancé, boyfriend or girlfriend, or the person you date seriously?" (EDQ89)

INDEPENDENT VARIABLES

STRESS AND PHYSICAL FITNESS TESTING IN THE 2005 DoD POPULATION SURVEY: IS THERE AN ASSOCIATION?

- stress-related questions (cont-)

"During the past 12 months,"—

- "how much did stress at work interfere with your ability to perform your military job?" (EDQ90)
- "how much did stress in your family life interfere with your ability to perform your military job?" (EDQ91)

- Participants' responses were grouped into
  - "None"
  - "A little, Some or A lot"

DEPENDENT VARIABLE

STRESS AND PHYSICAL FITNESS TESTING IN THE 2005 DoD POPULATION SURVEY: IS THERE AN ASSOCIATION?

- Result of fitness test
  - "Did you pass your most recent physical fitness test?"(EDQ 127)
- Participants' responses were grouped into "No" and "Yes"

COVARIATES

STRESS AND PHYSICAL FITNESS TESTING IN THE 2005 DoD POPULATION SURVEY: IS THERE AN ASSOCIATION?

Sociodemographic variables
- Age
- Gender
- Ethnicity
- Education
- Marital Status

Occupational variables
- Branch of service
- Rank/Paygrade
- Deployment past 3 years

Behavioral variables (No or Yes)
- Alcohol Use (Heavy Drinker)
- Tobacco Use (Recent Smoker)
- BMI (Obese)
- Activity (Routine Exerciser)

STATISTICAL ANALYSIS

STRESS AND PHYSICAL FITNESS TESTING IN THE 2005 DoD POPULATION SURVEY: IS THERE AN ASSOCIATION?

- Descriptive statistics-
  - weighted samples
- Univariate analysis-
  - outcome variable "fitness test result"
  - four stress-related predictor variables

STATISTICAL ANALYSIS

STRESS AND PHYSICAL FITNESS TESTING IN THE 2005 DoD POPULATION SURVEY: IS THERE AN ASSOCIATION?

- Multivariate analysis model-
- (covariates included if)
  - not highly correlated (>0.7) with other covariate, and
  - if they were significantly associated (p≤0.25) with the outcome in unadjusted regression analysis
- High correlation (0.67)-
  - education level and rank/paygrade

STATISTICAL ANALYSIS

STRESS AND PHYSICAL FITNESS TESTING IN THE 2005 DoD POPULATION SURVEY: IS THERE AN ASSOCIATION?

- Results-
  - odds ratios (OR) with respective 95% confidence intervals (95%CI)
- The statistical software package-
  - STATA © version 10.0 (STATA Corp., College Station, TX)

RESULTS

STRESS AND PHYSICAL FITNESS TESTING IN THE 2005 DoD POPULATION SURVEY: IS THERE AN ASSOCIATION?

Table 1. Main sample characteristics (n=12,860), 2005 DoD survey[a]

Characteristics	Unweighted sample (n=12,860)	Weighted sample (n=805,643)	Weighted Percentage(%)[b]
Passed last fitness test			
No	774	56,944	7.1
Yes	12,086	748,699	92.9
Stress experienced at work/military duties			
None	1,493	94,223	11.7
A little, Some or A lot	11,367	711,420	88.3
Stress experienced in family/personal life			
None	2,627	173,768	21.57
A little, Some or A lot	10,233	631,875	78.43
Work stress interfering with military job			
None	6,198	365,137	45.32
A little, Some or A lot	6,662	440,506	54.68
Family stress interfering with military job			
None	8,060	497,244	61.72
A little, Some or A lot	4,800	308,400	38.28

[a]The 2005 Department of Defense Survey of Health Related Behaviors among Active Duty Military Personnel.
[b]Percentages may not add up to 100 due to rounding.

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RESULTS

STRESS AND PHYSICAL FITNESS TESTING IN THE 2005 DoD POPULATION SURVEY: IS THERE AN ASSOCIATION?

Table 2. Sample covariates (n=12,860), 2005 DoD survey[a]

Characteristics	Unweighted sample (n=12,860)	Weighted sample (n=805,643)	Weighted Percentage (%)[b]
Sociodemographic			
Gender (Male)	9,857	692,391	85.9
Age (in years)			
17-20	982	109,169	13.5
21-25	3,210	254,201	31.5
26-34	3,538	252,803	31.4
35+	5,130	189,470	23.5
Ethnicity			
Non-Hispanic White	8,168	536,052	66.5
Non-Hispanic Black	1,947	130,928	16.2
Hispanic	1,503	68,367	8.5
Other	1,242	70,396	8.7
Education			
High School or less	3,223	262,122	32.5
Some College	5,567	354,655	44.0
College or More	4,070	188,867	23.4
Marital Status			
Not Married	4,733	358,629	44.5
Married, spouse not present	984	48,669	6.0
Married, spouse present	7,143	398,445	49.5

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RESULTS

STRESS AND PHYSICAL FITNESS TESTING IN THE 2005 DoD POPULATION SURVEY: IS THERE AN ASSOCIATION?

Table 2. Sample covariates (n=12,860), 2005 DoD survey[a] (CONTINUED)--

Characteristics	Unweighted sample (n=12,860)	Weighted sample (n=805,643)	Weighted Percentage (%)[b]
Occupational			
Branch of Service			
Army	2,897	257,299	31.9
Navy	3,492	206,385	25.6
Marine Corps	2,745	102,182	12.7
Air Force	3,726	239,777	29.8
Rank (Enlisted)	9,443	658,820	81.8
Deployment within past 3 years (Yes)	7,552	454,565	56.4
Behavioral			
Heavy Drinker[c] (Yes)	1,954	150,129	18.6
Recent Smoker[d] (Yes)	3,346	255,778	31.8
Obese[e] (Yes)	1,467	93,997	11.7
Routine Exerciser[f] (No)	2,818	177,079	22.0

[a]The 2005 Department of Defense Survey of Health Related Behaviors among Active Duty Military Personnel. [b] Percentages may not add up to 100 due to rounding. [c] Defined as consumption of more than four drinks on the same occasion at least once a week in the past 30 days. [d] Defined as having smoked a cigarette over the past 30 days. [e] Defined as BMI ≥ 30 kg/m[2] [f] Defined as having performed moderate or vigorous exercise for at least 20 minutes, a minimum of 3 times a week.

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RESULTS

STRESS AND PHYSICAL FITNESS TESTING IN THE 2005 DoD POPULATION SURVEY: IS THERE AN ASSOCIATION?

Table 3. Unadjusted association between the stress related variables and passing fitness test (n=12,860) in the 2005 DoD Survey [a]

Characteristics	%	Odds Ratio(95% CI)	p-value
Stress experienced at work/military duties			
None	93.8	1.00	
A little, some or a lot	92.8	0.86 (0.67, 1.11)	0.240
Stress experienced in family/personal life			
None	93.9	1.00	
A little, some or a lot	92.7	0.82 (0.61, 1.09)	0.168
Work stress interfering with military job			
None	94.8	1.00	
A little, some or a lot	91.4	0.59 (0.45, 0.77)	<0.001
Family/Personal Stress interfering with military job			
None	93.8	1.00	
A little, some or a lot	91.5	0.72 (0.60, 0.86)	0.001

[a] The 2005 Department of Defense Survey of Health Related Behaviors among Active Duty Military Personnel.

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RESULTS

STRESS AND PHYSICAL FITNESS TESTING IN THE 2005 DoD POPULATION SURVEY: IS THERE AN ASSOCIATION?

Table 4. Unadjusted association between covariates and passing fitness test (n=12,860) in the 2005 DoD Survey [a]

Characteristics	%	Odds Ratio (95% CI)	p-value
Sociodemographics			
Gender (Male)	93.53	1.00	
Female	89.25	0.57 (0.42, 0.79)	0.001
Age Category			
17-20	89.42	1.00	
21-25	92.36	1.43 (0.98, 2.08)	0.060
26-34	93.8	1.81 (1.28, 2.57)	0.001
35+	94.46	2.02 (1.30, 3.13)	0.002
Race/Ethnicity			
Non-Hispanic White	92.91	1.00	
Non-Hispanic Black	92.09	0.89 (0.70, 1.12)	0.306
Hispanic	94.12	1.22 (0.85, 1.76)	0.273
Other	92.93	1.09 (0.83, 1.44)	0.518
Marital Status			
Not Married	92.35	1.00	
Mar, spouse not present	90.82	0.82 (0.52, 1.29)	0.484
Mar, spouse present	93.71	1.24 (1.02, 1.50)	0.032

[a] The 2005 Department of Defense Survey of Health Related Behaviors among Active Duty Military Personnel. [b] Defined as: consumption of more than four drinks on the same occasion at least once a month in the past 30 days. [c] Defined as having smoked a cigarette over the past 30 days. [d] Defined as BMI ≥ 30 kg/m². [e] Defined as having performed moderate or vigorous exercise for at least 20 minutes, a minimum of 3 times a week.

RESULTS

STRESS AND PHYSICAL FITNESS TESTING IN THE 2005 DoD POPULATION SURVEY: IS THERE AN ASSOCIATION?

Table 4. Unadjusted association between covariates and passing fitness test (n=12,860) in the 2005 DoD Survey [a] (CONTINUED)--

Characteristics	%	Odds Ratio (95% CI)	p-value
Occupational			
Service			
Army	94.29	1.00	
Navy	91.78	0.68 (0.55, 1.02)	0.060
Marine Corps	96.03	1.46 (0.98, 2.19)	0.063
Air Force	91.14	0.62 (0.44, 0.89)	0.010
Paygrade (Enlisted)	91.69	1.00	
Officer	98.52	6.06 (4.01, 9.15)	<0.001
Deployment within past 3 years (No)	92.63	1.00	
Yes	93.17	1.08 (0.90, 1.31)	0.390
Behavioral			
Heavy Drinker[b] (No)	92.93	1.00	
Yes	92.93	1.00 (0.72, 1.39)	1.000
Recent Smoker[c] (No)	93.71	1.00	
Yes	91.27	0.70 (0.58, 0.85)	0.001
Obese (BMI≥30)[d] (No)	94.87	1.00	
Yes	78.25	0.19 (0.15, 0.24)	<0.001
Routine Exerciser[e] (No)	91.02	1.00	
Yes	93.47	1.41 (1.21, 1.65)	<0.001

[a] The 2005 Department of Defense Survey of Health Related Behaviors among Active Duty Military Personnel. [b] Defined as consumption of more than four drinks on the same occasion at least once a week in the past 30 days. [c] Defined as having smoked a cigarette over the past 30 days. [d] Defined as BMI ≥ 30 kg/m². [e] Defined as having performed moderate or vigorous exercise for at least 20 minutes, a minimum of 3 times a week.

RESULTS

STRESS AND PHYSICAL FITNESS TESTING IN THE 2005 DoD POPULATION SURVEY: IS THERE AN ASSOCIATION?

Table 5. Adjusted[a] association between stress exposures and passing fitness test (n=12,860), 2005 DoD Survey[b]

Characteristics	Odds Ratio	(95% CI)	p-value
Stress experienced at work/military duties			
None	1.00		
A little, some or a lot	0.86	(0.66, 1.11)	0.239
Stress experienced in family/personal life			
None	1.00		
A little, some or a lot	0.89	(0.65, 1.20)	0.431
Work stress interfering with military job			
None	1.00		
A little, some or a lot	0.69	(0.52, 0.90)	0.008
Family/Personal Stress interfering with military job			
None	1.00		
A little, some or a lot	0.80	(0.67, 0.97)	0.021

[a] Adjusted for the variables shown in Table 6. [b] The 2005 Department of Defense Survey of Health Related Behaviors among Active Duty Military Personnel.

DISCUSSION

STRESS AND PHYSICAL FITNESS TESTING IN THE 2005 DoD POPULATION SURVEY: IS THERE AN ASSOCIATION?

- **Associated with less likelihood of passing fitness test (i.e., failure):**
  - ***Work stress interfering with military job***
  - ***Family/Personal Stress interfering with military job***
- **No association established with fitness test result:**
  - ***Stress experienced at work/military duties***
  - ***Stress experienced in family/personal life***

DISCUSSION

STRESS AND PHYSICAL FITNESS TESTING IN THE 2005 DoD POPULATION SURVEY: IS THERE AN ASSOCIATION?

- Supporting research exploring the effects of the stress on physical fitness (though limited)
  - increase in tissue catabolism and physical fatigue associated with military training stress (Booth, et al., 2006)
  - presence of adverse obstetric outcomes from women with high levels of perceived personal stress(Haas and Pazdernik, 2006)

DISCUSSION

STRESS AND PHYSICAL FITNESS TESTING IN THE 2005 DoD POPULATION SURVEY: IS THERE AN ASSOCIATION?

STRENGTHS

- Large sample population
- Anonymous survey
- Cross-sectional study
  - more economical and feasible time period than a prospective study
- Previously validated survey

WEAKNESSES

- Limited to Military Population
  - Selected, may not be generalizable
  - "Selection Bias"
- Bias
  - Recall (survey)
  - Reporting (attribution?)
- Cross-sectional study
  - Temporal relationship cannot be established
- Stress not well defined

DISCUSSION

STRESS AND PHYSICAL FITNESS TESTING IN THE 2005 DoD POPULATION SURVEY: IS THERE AN ASSOCIATION?

- Temporal relationship (-)
  - Cross-sectional study design
- Strength of the association (+)
  - Odds Ratio

CONCLUSIONS

STRESS AND PHYSICAL FITNESS TESTING IN THE 2005 DoD POPULATION SURVEY: IS THERE AN ASSOCIATION?

- This study derived from the 2005 DoD Survey of Health Behaviors Among Military Personnel has yielded that there is an association between higher levels of perceived stress and lower fitness performance
  - Interventions addressing stress-related factor might prove beneficial in order to increase military readiness

ADDITIONAL RESEARCH

- Improve definition of stress (be more specific)
  - Work: deployment-related, supervisor interactions, co-worker relationships, excessive workload, and extended hours (>45 hours per week)
  - Personal: problems with the spouse or partner (i.e., marital strife), child conflict, illness in the family, financial concerns, and school/educational pressures

REFERENCES

- Complete list of references listed in original document.
  - Gordis, L. (2009) . From Association to Causation: Deriving Inferences from Epidemiologic Studies. In *Epidemiology*(4th ed.) (pp. 227-246). Philadelphia: Saunders Elsevier.
  - Gordis, L. (2009) . From More on Causal Inferences: Bias, Confounding, and Interaction. In *Epidemiology*(4th ed.) (pp. 247-263). Philadelphia: Saunders Elsevier.

The Association Between Stress and Physical Fitness Testing in the 2005 Department of Defense Population Survey

THANK YOU

The Error Rate of the Pushup Component of the USAF Fitness Assessment

19th Medical Operations Squadron (MDOS), Little Rock AFB, AR

Maj Eric Wilson

PURPOSE: To determine the Error Rate (ER) of the pushup component of the USAF Fitness Assessment. Numerous changes have recently occurred to the USAF's fitness program. With failure rates rising steadily, there is no reliability data to date on the test's most disputed content area. METHODS: Eight videos were made, each showing an individual performing one minute of maximum pushups while wearing one of the USAF fitness uniforms: t-shirt, long-sleeved shirt, sweatshirt, and jacket. Two videos were made for each subgroup. Ninety-two subjects undergoing PTL training (initial n=52; refresher n=40) viewed each of the eight videos once in random order and recorded the correct number of pushups performed. The Fitness AFI for pushup testing was read prior to viewing the videos. The primary investigator assessed the correct number of pushups by viewing the videos at ¼-speed with a grid overlapping the screen to assess elbow angles. RESULTS: ER was calculated for each subgroup (Mean, Standard Deviation, Range). The ER exceeded the number of pushups correctly performed in every subgroup. DISCUSSION: A trend in over-counting correct pushups was observed. Clothing had a significant effect on subject accuracy with t-shirts demonstrating the lowest ER compared to other subgroups. Exercise cadence, clothing variations, training and operational definitions are error sources contributing to the current grading criteria's inconsistent implementation. Air Force training should emphasize performance and recognition of appropriate form and a "tie goes to the runner" approach for testing.

Future considerations include redefining the operational definition and allotted time for the pushup test.

The Error Rate of the Pushup Component of the USAF Fitness Assessment

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Bottom Line Up Front

- The pushup assessment is not a reliable method for assessing muscular fitness of the upper body
- Sources of error include
  - Cadence of the exercise
  - Clothing worn
  - Training (graders and examinees)
  - Subjective grading criteria

Purpose

- To determine the error rate of the pushup component of the USAF Fitness Assessment.

Why?

- Numerous changes have been made to the Fitness AFI
- Failure rates are rising
- To date, there is no reliability data on the pushup assessment
- Pushup assessment is most difficult component to grade (a lot to observe as a grader)
  - Up position: elbows straight
  - Down position: elbows at least 90 degrees
  - Body must remain rigid during assessment
  - Incorrect pushup? Count last rep and explain what is being done incorrectly
  - Personnel <40 years old, average 1 pushup every
    - 1.05 seconds (male)
    - 1.30 seconds (female)

Exercise

Pushup Verbal Instructions: AFI 36-2905

- You must lower your upper body until your upper arm is at least parallel to the floor and elbows bent at 90 degrees before pushing back up to the starting position. If you do not come down that far the push-up will not count.
- Start in the up position with your elbows fully extended, feet no more than 12 inches apart, and your weight supported by your arms and toes. You must keep your back straight at all times and lower your upper body until your upper arm is at least parallel to the floor, then return to the up position with arms fully extended. This is one repetition.
- Resting must be done in the UP position.

15-Second Assessment: Exercise

- Pushup Video

How Many Correct Pushups?

Pushup Standards: Old vs. New

MALES

Age	Old Max	Age	New Max & *Min
<25	≥62	<30	≥67, *33
25-29	≥57		
30-34	≥52	30-39	≥57, *27
35-39	≥46		
40-44	≥40	40-49	≥44, *21
45-49	≥40		
50-54	≥39	50-59	≥44, *15
55+	≥35		
		60+	≥30, *14

FEMALES

Age	Old Max	Age	New Max & *Min
<25	≥42	<30	≥47, *18
25-29	≥41		
30-34	≥40	30-39	≥46, *14
35-39	≥30		
40-44	≥20	40-49	≥38, *11
45-49	≥18		
50-54	≥16	50-59	≥35, *9
55+	≥14		
		60+	≥21, *7

*Minimum scores = 5 points

New AFI: Consequences of "Poor" Score

- Unit CC may initiate (enlisted) or recommend (officer) administrative discharge of a member when:
  - 9.1.5.1.1. The member has accrued the second of two consecutive unsatisfactory fitness assessment scores; and
  - 9.1.5.1.2. The CC finds that the member failed to demonstrate significant improvement (as determined by the CC) despite the conditioning period; and
  - 9.1.5.1.3. Evaluation by a military health care provider has ruled out medical conditions precluding the member from achieving a passing score.

Methods: Overview

- Subjects undergoing Physical Training Leader (PTL) training were shown 8 videos of Airmen performing 1 minute of maximum effort pushups.
- They had to identify the number of correct pushups performed in each video.
- The fitness AFI on appropriate pushup assessment was read to each group prior to data gathering.
- Videos were shown in random order for each group.

Methods: Videos

- 8 videos were made
- Each showed 1 minute of maximum effort pushups
- Camera at head height in the "up" position of the test
  - Distance from camera to subject standardized
- 4 subgroups of videos, 2 for each variation in uniform
  - T-Shirt
  - Long-Sleeve Shirt Under T-Shirt (UnderArmor, etc)
  - Sweatshirt
  - Jacket

Methods: Sample

- n=92 undergoing Physical Training Leader (PTL) training
  - n=52 initial training
  - n=40 refresher training
- Gender: 52% Male
- Mean Age: 27
- Average Rank: Senior Airman (E-4)
- Mean Time in Service: 43 months
- Subjects did not know this assessment was part of a study. They believed it was part of their PTL training.

Methods

- Determining correct number of pushups per video
- Primary investigator assessed each video at 25% speed
- Grid overlaying screen to measure elbow angles in the up and down positions
- All eight videos watched consecutively and scores recorded on separate piece of paper
- Each video was scored 5 times
- Number of "correct" pushups was mean of 5 viewings
  - 100% agreement

Study Data

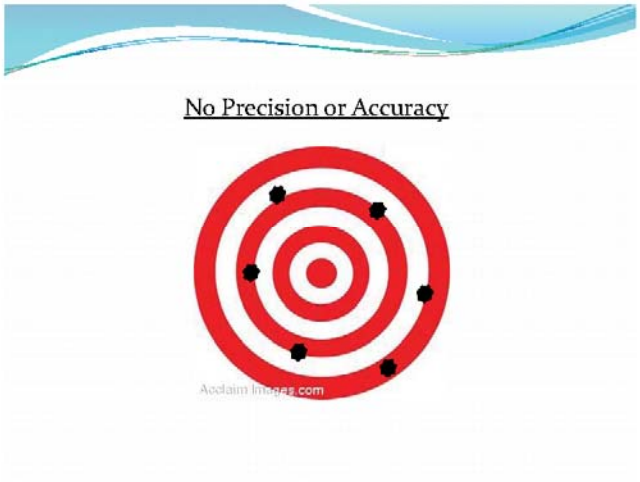
Clothing	Actual Count	Mean Error	Std Dev Error	Range Error
Jacket A	2	19.82	14.56	0 - 40
Jacket B	18	8.92	8.82	0 - 42
Longsleeve A	14	8.42	9.53	0 - 34
Longsleeve B	4	10.13	6.55	0 - 25
Sweatshirt A	1	32.93	9.09	3 - 38
Sweatshirt B	2	21.25	11.02	0 - 43
T-shirt A	4	6.10	8.73	0 - 31
T-shirt B	18	5.05	5.11	0 - 29

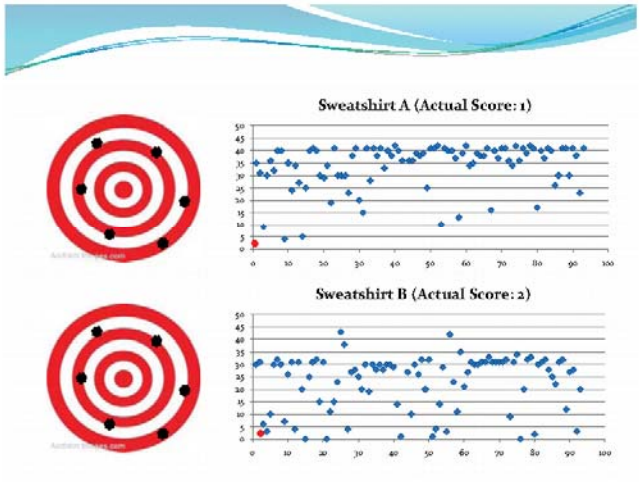
Precision vs. Accuracy

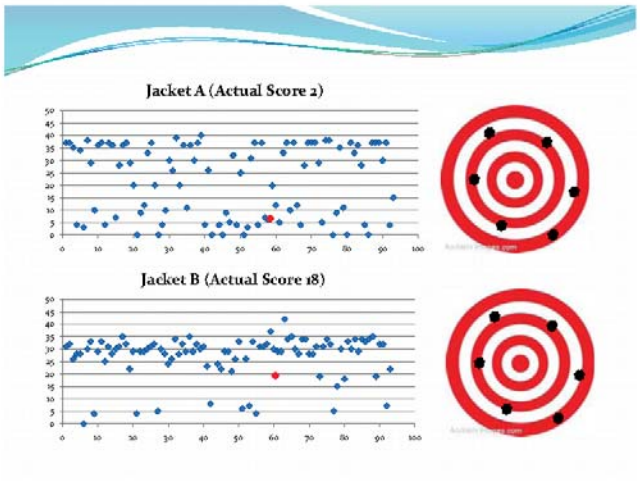
- Precision: reproducible performance
  - Marksmanship: how close you can group your shots
- Accuracy: quality of being near to the true value
  - Marksmanship: how close to the bulls-eye you get your shots

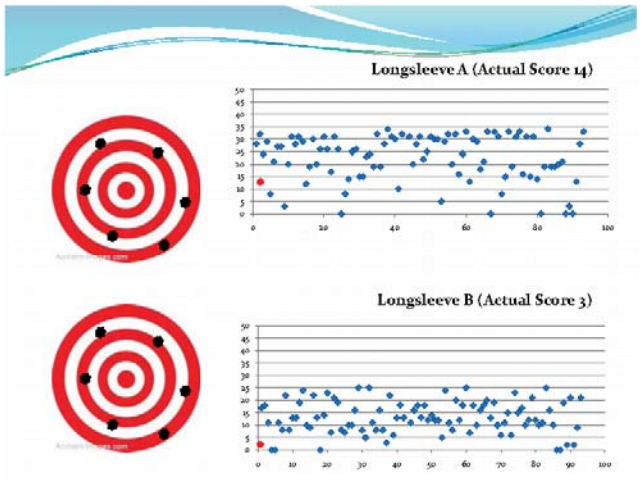
Accuracy & Precision

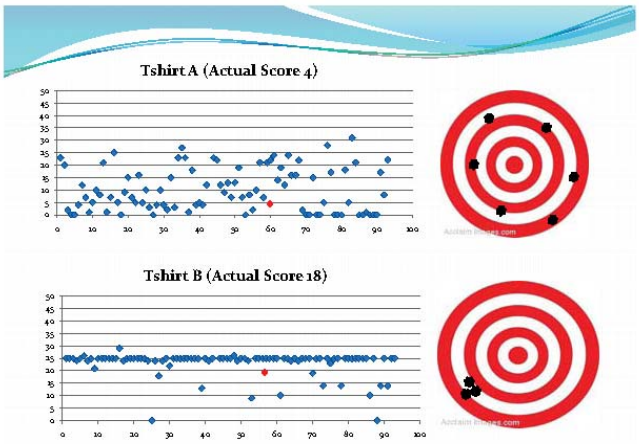
Precision without Accuracy


No Precision or Accuracy


Sweatshirt A (Actual Score: 1)
Sweatshirt B (Actual Score: 2)


Jacket A (Actual Score 2)
Jacket B (Actual Score 18)


Longsleeve A (Actual Score 14)
Longsleeve B (Actual Score 3)



Results

- No significant difference between new PTLs and experienced PTLs
- All groups have positive mean error rate
- All groups have large range and std dev of error
- Clothing significantly decreased accuracy of grading

Interpretation - Experience

- Experience was not a significant factor in accuracy of grading
- Experienced PTLs demonstrated higher error rates in 5/8 groups
- Over Counting: "Good Enough" vs "Correct Form"

Interpretation - Clothing

- Clothing had a significant effect on the PTL's ability to accurately grade
- Groups with T-shirt demonstrated lowest mean error rate of 5.05 and 6.10
- AFI allows large variation in clothing

Interpretation - Training

- Training not adequate to accurately grade
- Average mean error varied from 5.05 to 32.93
- Average range error varied from 25.0 to 42.0
- Current method of training/grading may be unrealistic

Possible Cause of Errors

- Speed vs Form
  - Push ups are done so fast causing poor form (examinee)
  - Difficult to accurately determine correct form (grader)
- Clothing Variation
  - Bulky clothing creates difficulty for grader to identify correct form
- Current grading criteria
  - Too subjective to be implemented and graded consistently

Possible Solutions: Short-Term

- Shift Focus through training
  - Away from "maximum pushups able to perform" to
  - Maximum pushups grader can accurately count
- Fit Test preparation commensurate with UCI
- Educate Airmen, PTLs, and FACs of errors inherent with current method
  - Commanders advocate "tie goes to examinee" standard
- Stress importance of testing in T-shirts
  - Bulkier clothing = harder for grader

Possible Solutions: Long-Term

- Use of similar videos during FAC/PTL training
- Graders must demonstrate sufficient & sustained levels of accuracy in grading the pushup component

Possible Solutions: Long-Term

- Increase time allotted to 90 seconds
- Will continue to assess immediate & short-term non-oxidative energy systems without contribution of aerobic-oxidative
- Decrease overall cadence of exercise which will improve grader accuracy

Possible Solutions: Long-Term

Change operational definition of pushup assessment

- American College of Sports Medicine (ACSM)
  1. Start in "down position"
  2. 1 rep = raise body until elbows extended, lower body until chin touches ground (stomach may not touch)
  3. Back must be straight at all times
  4. Maximum consecutive reps without rest
  5. Test stopped when the examinee:
     1. Strains forcibly or
     2. Cannot maintain proper technique within 2 reps
  6. Modification for females: pivot point on knees vs. toes

Possible Solutions: Long-Term

American College of Sports Medicine Scoring Criteria

- ***Modifications may be required for USAF use***

Category	AGE									
	20-29		30-39		40-49		50-59		60-69	
Gender	M	F	M	F	M	F	M	F	M	F
Excellent	36	30	30	27	25	24	21	21	18	17
Very Good	35	29	29	26	24	23	20	20	17	16
	29	21	22	20	17	15	13	11	11	12
Good	28	20	21	19	16	14	12	10	10	11
	22	15	17	13	13	11	10	7	8	5
Fair	21	14	16	12	12	10	9	6	7	4
	17	10	12	8	10	5	7	2	5	2
Needs Improvement	16	9	11	7	9	4	6	1	4	1

Causes of Errors vs. Solutions

Solutions	Causes of Errors		
	Exercise cadence too fast	Clothing variations	Grading criteria too subjective
Training Examinee	✓		✓
Training Grader	✓		✓
T-shirt use		✓ ✓	
90 sec time limit	✓		
ACMS guidelines	✓ ✓		✓ ✓

Limitations of Study

- Unequal group numbers.
- Number of push ups done differs between conditions.
- Number of push ups done correctly and incorrectly differ between conditions
- Different subjects used for each conditions.

Future Directions of Study

- Future study changes:
  - Equal number correct and incorrect pushups done in random manner for each condition.
  - Intra-rater and inter-rater grading will be performed to determine consistency.
  - Larger number of subjects
- Possible future study changes/additions:
  - Use of model with different body types to determine effect
  - Use of Male and female models to determine effect

Questions?

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Effects of sit-up training versus core stabilization exercises on sit-up performance: A cluster randomized trial

US Army-Baylor Doctoral Program in Physical Therapy

Lt Col John Childs, Deydre S. Teyhen, Timothy M. Benedict, Jamie B. Morris, Andrew D. Fortenberry, Rene M. McQueen, Jane B. Preston, Alison C. Wright, Jessica L. Dugan, Steven Z. George

PURPOSE: Core stabilization exercises target abdominal and trunk muscles without the excessive loading that occurs during sit-ups. However, core stabilization exercise programs (CSEP) have not been widely adopted in the U.S. Army because of the perceived deleterious impact they would have on performance during the Army Physical Fitness Test. The purpose was to determine whether performing CSEP in lieu of sit-ups during physical training would have detrimental effects on sit-up performance and passing rates on the fitness test. METHODS: Soldiers (N=2616) between 18-35 years of age were randomized to receive a traditional exercise program (TEP) with sit-ups or CSEP. Subjects with a previous history of low back pain or other injury precluding participation in training were excluded. Training programs were completed four times per week over 12 weeks. Performance was assessed at baseline and 12 weeks. RESULTS: Both groups demonstrated significant improvements in sit-up performance and overall fitness scores over time ($P<0.001$). There was no significance between group differences in overall fitness scores ($P=0.142$) or sit-up performance ($P=0.543$). However, CSEP resulted in a significant improvement in sit-up passing rates by 5.6% compared to 3.9% for the TEP group ($P=0.004$). CONCLUSION: There was a small but significantly greater increase in sit-up pass rate in the CSEP (5.6%) versus the TEP group (3.9%). Incorporating CSEP into Army physical training does not increase the risk of suboptimal performance on the Army's fitness test and may offer a small benefit for improving sit-up performance.

Background

- Low back pain (LBP) is one of the most common forms of chronic pain (Martin 2008, Luo 2004, Stewart 2003)
- Leading factor for medical board processing in the military (Songer 2000)
- High cost of LBP
  - Lifetime compensation cost (van Tulder 1997)
  - High tax payer dollars (Kaufman 2000)
  - Decrease mission readiness (Knapik 1993, Jones 1999)

Background

- Traditional sit-ups may increase the risk of injury and development of LBP (Axler 1997, McGill 1988, Nachemson 1999)
- Evidence supports use of core stabilization exercise (CSEP) to potentially decrease LBP frequency and pain and increase performance (Hicks 2005, Hides 2001, O'Sullivan 1997)
  - Decreasing recurrence of LBP
  - Decreasing LE injury incidence (Leetun 2004)

Background

- U.S. Army Physical Fitness School is implementing a "4 for the Core" exercise program as part of unit physical training
- Concerns with decrease sit-up performance on Army Physical Fitness Test (Childs 2009) and increase risk of other musculoskeletal (MSK) injuries with CSEP

Purpose

- Explore the short-term effects of a core stabilization exercise program (CSEP) without sit-up training compared to a traditional exercise program (TEP) on MSK injury incidence and work restriction in US Army physical training.
- We hypothesized there would no difference in injury rates between CSEP and TEP

Methods

Subjects

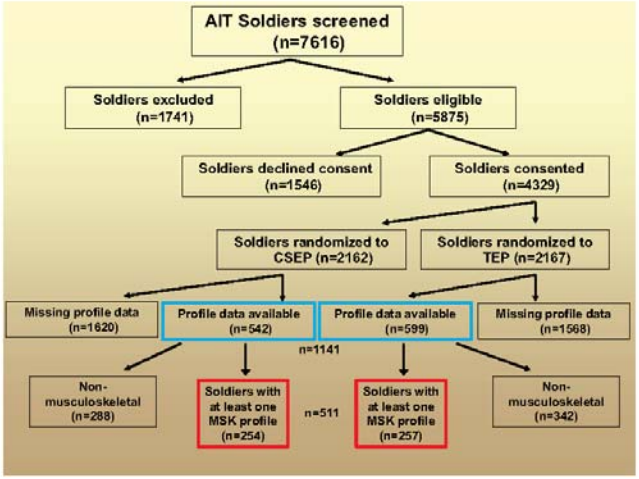
- 4329 Advanced Individual Training (AIT) US Army Soldiers
  - 232nd Medical Battalion
  - 68W students
  - Fort Sam Houston, TX
- Inclusion criteria
  - Soldiers between 18-35 years of age (or 17 year old emancipated minor)
  - Complete injury data available (n=1,141)
  - English speaking and reading

Exclusion Criteria

- Prior history of LBP with all of the following:
  - Limited work or physical activity
  - Duration > 48 hours
  - Caused individual to seek medical care
- Currently seeking medical care for LBP
- Previous medical history including surgery for LBP
- Currently unable to participate in unit exercise due to injury in foot, ankle, knee, hip, neck, shoulder, elbow, wrist, or hand
- History of fracture (stress or traumatic) in hip and/or pelvis
- Pregnant
- Transferred from another AIT Company

Cluster Randomization

- Cluster randomization (Olsen 2005, Ornstein 2004, Watson 2004)
  - Company units randomly assigned
  - Determined prior to recruitment
  - Balanced
- Individual randomization was not utilized
  - Detract from unit cohesion
  - Inevitable contamination of treatment groups
  - Burdensome for company instructors



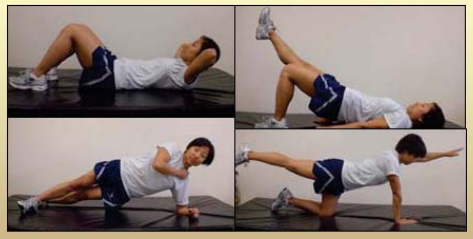
Exercise Programs

- Soldiers randomized to complete CSEP (n=542) or TEP (n=599)
- Performed at unit physical training
- Frequency: 5 minutes/day, ≥ 4 days/week
- Led by Company instructors
  - Company instructors were provided training
  - Study personnel observed training weekly
  - Weekly meetings with Company instructors

Traditional Exercise Program (TEP)

- Commonly performed exercises in the military for physical training
- Targeted muscles: Rectus abdominus, internal and external oblique, and hip flexor muscles
- Quick, high-load, high repetition

Core Stabilization Exercise Program (CSEP)



- Evidence-based
- Targeted muscles: transverus abdominus, multifidi, erector spinae, quadratus lumborum, and hip flexor musculature
- Slow, low-load, minimal trunk motion

Physical Profile

- DA Form 3349, AR 40-501
- Physical Profiling is a system of classifying individuals according to functional abilities (Ch 7 AR 40-501)
- Profiles assess a Soldier's medical condition and functional activity limitations
- Allows a reduction of aggravating activities for specific period of time

Results

Data Analysis

- Descriptive statistics calculated for background information
- Differences in the percentage of MSK injuries examined with chi-square
- Independent samples t-tests used to examine differences in the number of work restricted days
- Alpha set to 0.05 a priori

Data Analysis

- Independent variable: exercise group
  - TEP
  - CSEP
- Dependent variables:
  - Rate of musculoskeletal injury
  - Incidence of musculoskeletal injury
  - Duration of injury
- $\alpha = 0.05$

Variable	All Soldiers (n=1141)	CSEP (n=542)	TEP (n=599)	P-value
Age (years)	22.7 (4.6)	22.6 (4.5)	22.7 (4.7)	0.745
Gender (% male)	60.9	60.1	61.6	0.615
BMI (kg/m2)	24.9 (3.6)	24.8 (3.2)	24.9 (3.9)	0.538
Complete profile data available	26.4	25.2	27.7	0.059
Currently smoke (%)	41.2	42.1	41.6	0.776
Previous routine exercise (%)	42.7	47.2	44.9	0.127
Education, some college (%)	56.3	56.6	55.9	0.808
Income >20,000 (%)	55.2	51.8	58.3	0.029
Previous Profile (%)	32.4	33.0	32.7	0.818

Table 1: Demographics of all Soldiers who sustained at least one injury resulting in limited duty days (n=1141)

Injury Type	CSEP (n=542)	TEP (n=599)	P-value
Musculoskeletal (Any)	46.9% (n=254)	42.9% (n=257)	0.179
Low back	13.3% (n=72)	11.0% (n=66)	0.241
Lower extremity	31.5% (n=171)	30.7% (n=184)	0.762
Upper extremity	6.1% (n=33)	4.5% (n=27)	0.232

Table 2: Percentage of all injured who sustained at least one MSK injury that resulted in work restrictions (n=511)

Injury Type	CSEP Mean (SD) (95% CI) (n=254)	TEP Mean (SD) (95% CI) (n=257)	Difference Mean (SD) (95% CI)	P-value
Musculoskeletal (Any)	1.3 (0.5) (1.19-1.32)	1.2 (0.5) (1.2-1.3)	-0.04 (0.1) (-0.1-.1)	0.410
Low back	0.3 (0.6) (0.3-0.4)	0.3 (0.5) (0.2-0.3)	0.1 (0.1) (-0.2-0.0)	0.247
Lower extremity	0.8 (0.6) (0.7-0.9)	0.8 (0.61) (0.7-0.9)	0.0 (0.1) (-0.1-0.1)	0.590
Upper extremity	0.1 (0.3) (0.09-0.17)	0.1 (0.3) (0.07-0.15)	-0.0 (0.0) (-0.1-0.0)	0.474

Table 3: Mean number of MSK injury profiles per injured Soldier per body region (n=511)

Injury Type	CSEP Mean (SD) (95% CI) (n=542)	TEP Mean (SD) (95% CI) (n=599)	Difference Mean (SD) (95% CI)	P-value
Musculoskeletal (Any)	20.4 (16.9) (18.3-22.4)	21.4 (24.7) (18.4-24.5)	1.1 (1.9) (-2.6-4.8)	0.568
Low back	4.2 (8.0) (2.4-6.1)	8.3 (14.5) (4.7-11.8)	4.0 (2.0) (0.02-8.0)	0.049
Lower extremity	19.5 (15.6) (17.1-21.9)	20.0 (23.8) (16.6-23.5)	0.52 (2.2) (-3.7-4.8)	0.81
Upper extremity	24.0 (23.1) (5.8-32.2)	19.5 (17.0) (12.8-26.2)	-4.48 (5.4) (-15.2-6.2)	0.406

Table 4: Mean number of limited duty days per injured Soldier per body region (n=511)

Discussion & Conclusions

Discussion

- Of 1141 Soldiers who had complete injury data available for analysis, 511 (44.8%) Soldiers experienced a MSK injury during training that resulted in work restrictions
- Overall MSK injury rates and incidence between the groups as a whole and by body region were similar (P>.05)
  - Higher risk of LE injuries in both groups

Discussion

- No difference in the number of work restricted days for MSK injuries overall or specific to the UE or LE (P>.05)
- Soldiers in the TEP group who experienced a LB injury experienced more work restricted days (TEP=8.3±14.5; CSEP=4.2±8.0, P=0.049)

Discussion

- Study Limitations
  - Inconsistent reporting of data for consented Soldiers
    - However, reporting rates similar between groups (P>.05)
  - Effects of previous MSK injuries prior to basic training
  - Number of days from start of AIT to first profile
- Future Research
  - Long term effects of CSEP on all MSK injuries
  - Military versus civilian population

Conclusion

- CSEP does not increase incidence, rate, or duration of MSK injuries
- Incorporation of CSEP has potential to reduce limited duty days due to LBP
- CSEP is a low risk intervention for Army physical training

Funding & Approval

- Funding:
  - Congressionally Directed Peer-Reviewed Medical Research Programs
  - 4 year - $1 million dollar grant
- Approval:
  - Brooke Army Medical Center (Feb 2006)
  - University of Florida (June 2006)
- Registration:
  - http://clinicaltrials.gov
  - NCT00373009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Jessica Neff
232nd Medical Battalion
Brett Neilson, PT


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John May, Elizabeth Sonnenberg
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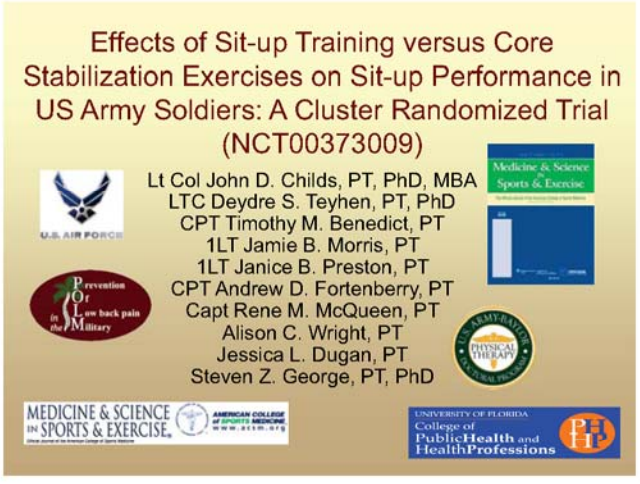
Monica Montanez


Questions?


Effects of Sit-up Training versus Core Stabilization Exercises on Sit-up Performance in US Army Soldiers: A Cluster Randomized Trial (NCT00373009)
Lt Col John D. Childs, PT, PhD, MBA
LTC Deydre S. Teyhen, PT, PhD
CPT Timothy M. Benedict, PT
1LT Jamie B. Morris, PT
1LT Janice B. Preston, PT
CPT Andrew D. Fortenberry, PT
Capt Rene M. McQueen, PT
Alison C. Wright, PT
Jessica L. Dugan, PT
Steven Z. George, PT, PhD

Background

- Low back pain (LBP) is one of the most common forms of chronic pain (Martin 2008, Luo 2004, Stewart 2003)
- Treatment and prevention exercise programs for LBP reported in the literature commonly involve muscles involved in core stabilization (Hicks 2005, Hides 2001, O'Sullivan 1997)
- Evidence supports use of CSEP to potentially decrease LBP and increase performance (Hicks 2005, Hides 2001, O'Sullivan 1997)

Background

- Traditional sit-ups may increase the risk of injury and development of LBP (Axler 1997, McGill 1988, Nachemson 1999)
- Perceived deleterious impact on sit-up performance in the Army Physical Fitness Test (APFT) if units adopt a CSEP
  - Preliminary research on West Point cadets (Baxter 2003)
- Need for assessment of CSEP impact on sit-up performance

Purpose

- Determine whether performing CSEP in lieu of sit-ups during a 12-week training period has detrimental effects on performance of sit-ups and overall fitness as measured by the APFT
  - We hypothesized that there would be no differences in APFT sit-up scores or overall passing rates based on whether subjects performed a TEP or CSEP.

Methods

Subjects

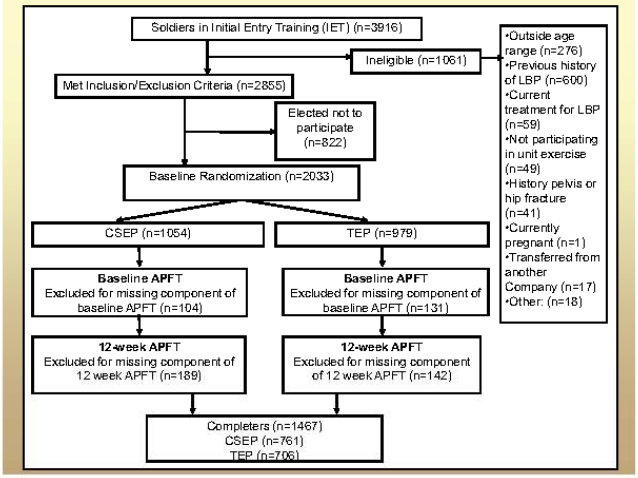
- Advanced Individual Training (AIT) US Army Soldiers
  - 232nd Medical Battalion
  - Combat medics
  - Fort Sam Houston, TX
- Mean age: 21.9 ± 4.3 yrs (Range 17-35)
- Inclusion criteria
  - 18-35 years of age (or 17 year old emancipated minor)
  - English speaking and reading

Exclusion Criteria

- Prior history of LBP with all of the following:
  - Limited work or physical activity
  - Duration > 48 hours
  - Caused individual to seek medical care
- Currently seeking medical care for LBP
- Previous medical history including surgery for LBP
- Currently unable to participate in unit exercise due to injury in foot, ankle, knee, hip, neck, shoulder, elbow, wrist, or hand
- History of fracture (stress or traumatic) in hip and/or pelvis
- Pregnant
- Transferred from another AIT Company

Cluster Randomization

- Individual randomization was not utilized
  - Detract from unit cohesion
  - Inevitable contamination of treatment groups
  - Burdensome for drill instructors
- Cluster randomization (Olsen 2005, Ornstein 2004, Watson 2004)
  - Company units randomly assigned
  - Determined prior to recruitment
  - Balanced



Exercise Programs

- 2 exercise programs: TEP & CSEP
- Performed at unit physical training
- Frequency: 5 minutes/day, 4 days/week
- Led by Drill Sergeants
- Drill Sergeants were provided training and training aids by study personnel
- Study personnel routinely observed training
- Weekly meeting with cadre to answer questions/concerns

Traditional Exercise Program

- Commonly performed exercises in the military for physical training
- Targeted: Rectus abdominus, internal and external oblique, and hip flexor muscles
- Quick, high-load, high repetition

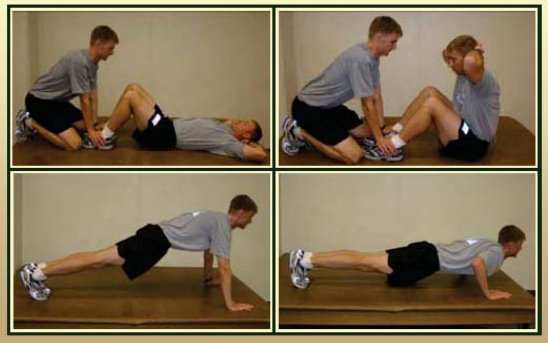
Core Stabilization Exercise Program (CSEP)

- Evidence-based
- Targeted: transverus abdominus, multifidi, erector spinae, quadratus lumborum, and hip flexor musculature
- Slow, low-load, minimal trunk motion

APFT

- Events:
  - Maximum push-ups in 2 minutes
  - Maximum sit-ups in 2 minutes
  - 2 mile run
- Scores adjusted for age and sex
- Minimum passing score: 60% in each event
- Cadre that were not directly involved in this study assessed Soldier performance

APFT sit-ups and push-ups



Data Analysis

- Descriptive statistics
- 2×4×2 repeated-measures ANOVA with pairwise comparisons using the Bonferroni inequality
- Independent variables
  - Exercise group (TEP and CSEP)
  - Quartile (0-25%, 26-50%, 51-75%, 76-100%)
  - Time (baseline, 12 wks)

Data Analysis

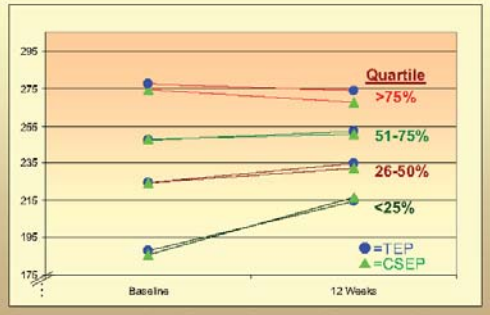
- APFT sit-up score (x/100)
  - APFT sit-up pass rate
  - Overall APFT score
- Number Needed to Treat
- $\alpha = 0.05$

Results

Demographic Results

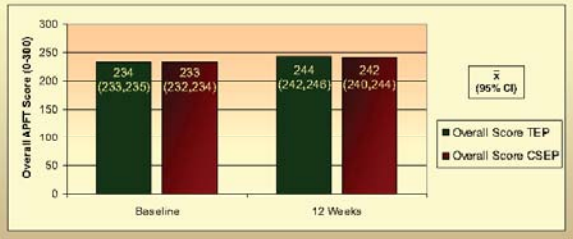
Variable	All Soldiers (n=1467)	CSEP (n=761)	TEP (n=706)	Significance
Age (years)	21.9 (4.3)	21.9 (4.4)	21.9 (4.1)	0.944
Gender (% male)	73.3	72.5	74.2	0.466
BMI (kg/m^2)	24.7 (4.0)	24.8 (4.3)	24.7 (3.7)	0.750
Currently smoke (%)	32.8	31.6	34.0	0.333
Previous routine exercise (%)	54.3	55.4	53.3	0.422

Overall APFT Score by Quartile



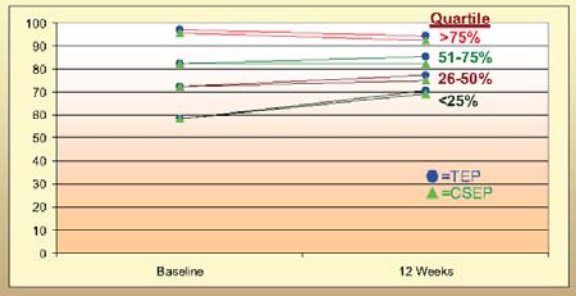
There was no significant Group x Quartile x Time interaction (p = 0.164)

Overall APFT Score by Group At Baseline and 12 Weeks



There was no significant difference between groups in overall APFT scores at 12 weeks (p=0.142)

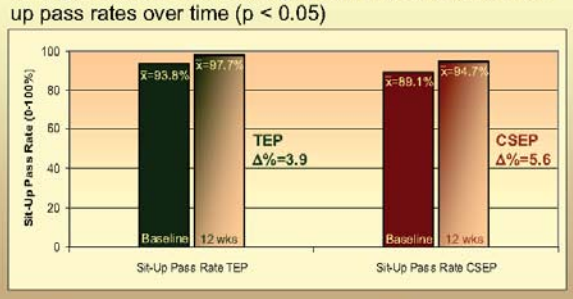
Sit-Up Score by Quartile



There was no significant Group x Quartile x Time interaction (p = 0.543)

Change in Sit-Up Passing Rate

Both groups demonstrated significant improvements in sit-up pass rates over time (p < 0.05)



CSEP improved at a greater rate (p < 0.05)

APFT Results

- There was a significantly greater increase in sit-up pass rate in the CSEP (5.6%) versus the TEP (3.9%) group (p < 0.05)
- The NNT to prevent 1 additional failure on the sit-up component of the APFT with CSEP was 56
- Both groups performed sit-ups outside of unit PT at equal rates (TEP: 69.5% and CSEP: 65%, p = 0.067)

Discussion & Conclusions

Discussion

- CSEP did not have a detrimental impact on APFT scores
- Sit-up scores and pass rates improved significantly in TEP and CSEP
  - CSEP improved sit-up pass rate by 5.6%
- No increase in APFT failure rate with CSEP
- CSEP is a low risk intervention to incorporate into Army Physical Training

Discussion

- Study Limitations
  - Sit-up exposure in CSEP group
  - Ceiling effects for APFT
  - Relevance to Soldiers with high levels of physical fitness
- Future Research
  - Subjects with high level of fitness
  - Prior history of LBP

Conclusions

- CSEP does not increase risk of sit-up rate failure on the APFT
- Can incorporate core stability exercises into the Army physical fitness program without detriment to APFT scores
- A 400 Soldier AIT company performing CSEP would progress 7 more Soldiers from fail to pass on the sit-up component than TEP

Funding & Approval

- Funding:
  - Congressionally Directed Medical Research Program (Peer-Reviewed Medical Research Program)
- Approval:
  - Brooke Army Medical Center (Feb 2006)
  - University of Florida (June 2006)
- Registration:
  - http://clinicaltrials.gov
  - NCT00373009

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East Tennessee State University

Alison Wright, Melissa Ogle, Alexandra Gentles

University of Colorado at Denver and Health Sciences Center

John May, Elizabeth Sonnenberg

Texas State University-San Marcos

Monica Montanez

Questions?

Embedded Fragments - A Unique Exposure Situation and Concerns of Possible Health Effects

Armed Forces Institute of Pathology

Jose A. Centeno

BACKGROUND: The majority of modern war wounds are characterized by high-energy blast injuries containing a wide range of retained foreign materials of a metallic or composite nature. Health risks of retained fragments such as local or systemic toxicities, and delayed outcomes such as foreign body reactions or malignancies, are dependent of the chemical composition of the fragments and need to be further understood. Information obtained by chemical analysis of excised fragments can be used to guide clinical decisions regarding the need for fragment removal, to develop therapeutic interventions, and to better manage potential future medical problems arising from retained fragment related injuries. OBJECTIVES: The objective of this study is to define the chemical composition of retained embedded fragments removed from injured military personnel, and to relate results to histological findings in tissue adjacent to fragment material. RESULTS: Most fragments were obtained from penetrating wounds sustained to the extremities, particularly soft tissue injuries. The majority of the fragments were composed of single metals such as iron, copper, and aluminum with traces of antimony, titanium, uranium, and lead. CONCLUSIONS: The present study provides a systematic approach for obtaining a full chemical characterization of retained embedded fragments. Given the vast number of combat casualties with retained fragments, it is expected that fragment analysis will have significant implications for the optimal short and long-term care of wounded service members.

Armed Forces Institute of Pathology

AFIP Embedded Metal Fragment Registry

Embedded fragments present unique exposure situations and concerns of possible health risks

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August 24,26, 2010
Arlington, VA

Disclaimers:

The opinions presented are the private views of the speaker, and should not be construed as official or representing the views of the Department of Army, the Department of Defense, the Armed Forces Institute of Pathology, or other federal agencies.

Certain equipment and instruments or materials are identified in this presentation to adequately specify the experimental details. Such identification neither implies recommendation by the Department of the Army, nor that the materials are necessarily the best available for the purpose.

Health Effects from Embedded Fragments

Outline

- Background on embedded fragments
- History of AFIP research on embedded DU and metal fragments
- DoD Policy (HA-07-029)* on embedded metal fragments



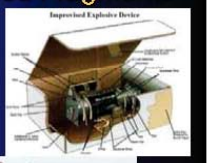
*HA Policy 07-029: Analysis of Metal Fragments removed from DoD Personnel, 18 Dec 2008

Photos: Courtesy of Associated Press

Health Effects from Embedded Fragments

Background

- Traumatic injury via contact with improvised explosive devices (IEDs) are of major concern in Iraq and increasingly in Afghanistan
  - ✓ More than 5,000 soldiers with traumatic injuries may have retained embedded fragments;
  - ✓ IEDs are packed with heterogenous material including metals, plastics, glass, etc.




Photos: Courtesy of Associated Press

Health Effects from Embedded Fragments
Background (cont.)

- Animal studies and human case reports suggest that retained fragments, over time, may interact with body tissues resulting in local and systemic effects;
  - ✓ Evidence from the VA DU Program, medical devices*, and fragment literature;
  - ✓ Known renal, reproductive and neurological effects of metals (e.g., Pb, Cd, Ni, Mn, Cu)
- Some metals used in weapons are potentially harmful as retained metal fragments by virtue of their toxicity;
  - ✓ New studies of metal implanted in animals with tungsten alloys (W:Ni:Co alloys) demonstrating rapid carcinogenesis
- Current policies/procedures are inadequate for assessing and managing casualties with all types of retained metal fragments;
- All metal fragments that are removed from Service members should be sent for chemical characterization and archival (HA Policy No. 07-029)**.

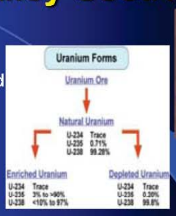
* International Agency for Research on Cancer, "Surgical implants and other foreign bodies", Vol. 74, 1999.
**HA Policy 07-029: Analysis of metal fragments removed from DoD personnel, 18 Dec 2008.

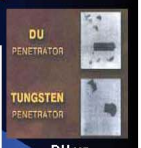
Metals of Military Relevance
(Depleted Uranium)

- Metals of interest: Pb, Cu, Mn, DU, W, Ni, As, Sb, Sn
- Depleted uranium (DU) is considered the "gold standard" for armor penetrating munitions.
- However, continuing uncertainty about long-term DU health effects and widespread public efforts to ban use of DU in munitions have led to a search for substitutes for DU in munitions.
- Certain tungsten alloys are leading candidates.

Why is DU Alloy Used?

- Dense (19 g/cm^3) – high inertia
- Metallurgical and chemical properties when alloyed with Ti and other metals
- Availability (By-product)
- About 50% less radioactive than natural U.





DU vs Tungsten

Military use of depleted uranium:

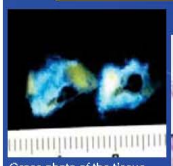
Heavy armor
Tanks – variant of Abrams M1A1

120 mm anti tank rounds

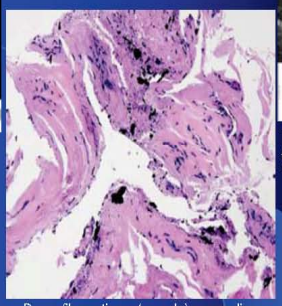
A10 Warthog

Photos: Courtesy of Associated Press

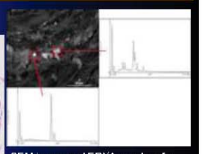
Embedded DU fragments in tissue removed from shrapnel wounds



Gross photo of the tissue removed from the shoulder. The black area in the center is the foreign body-depleted uranium remnant.

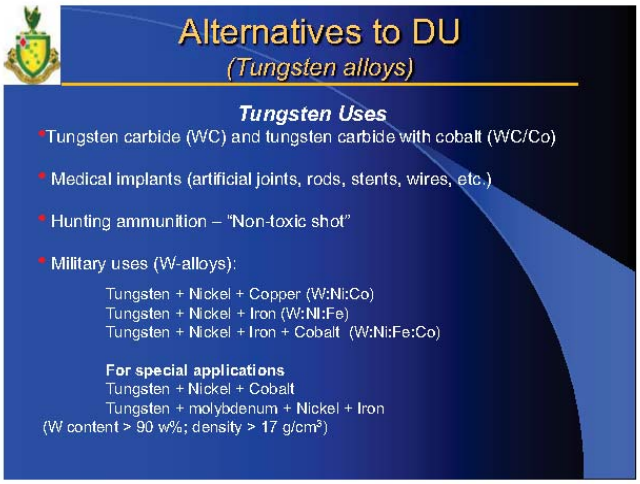


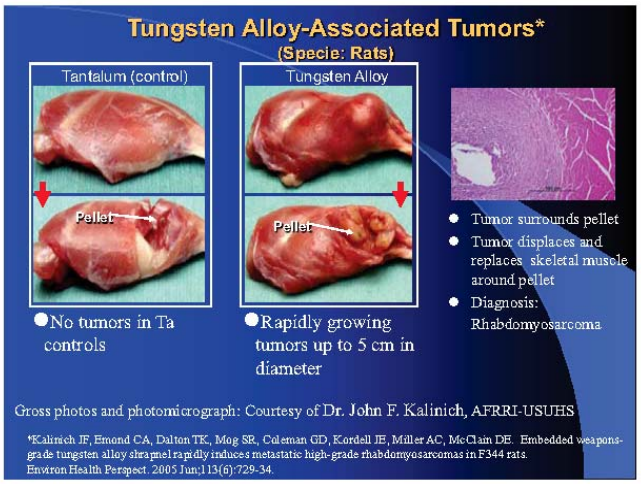
Dense fibrous tissue (capsule) surrounding the shrapnel composed of pausicellular fibrous tissue, and scattered fragments of black material.

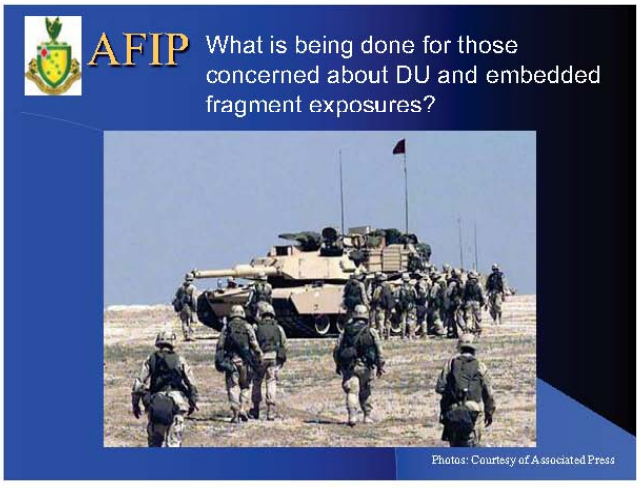


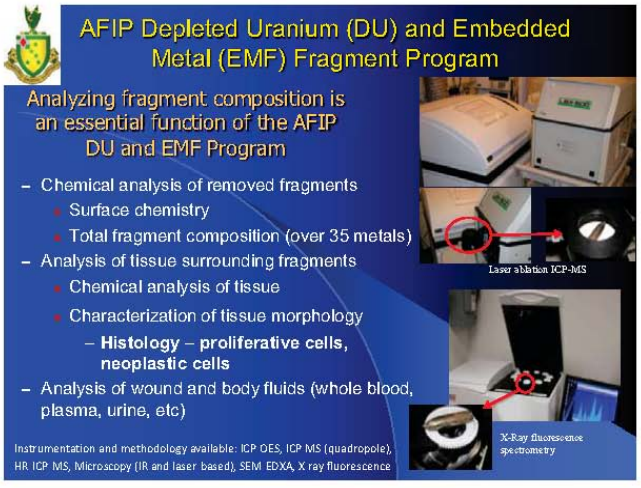
SEM image and EDXA spectra of a region of tissue with granular black fragments demonstrating the presence of uranium and titanium.

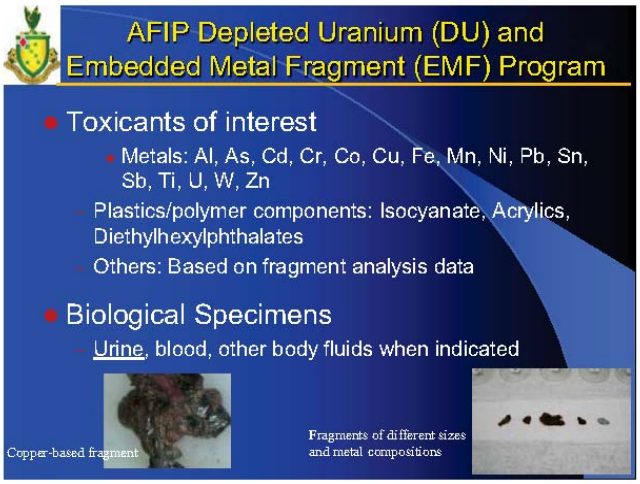
Photos and photomicrographs: Jose A. Centeno, AFIP

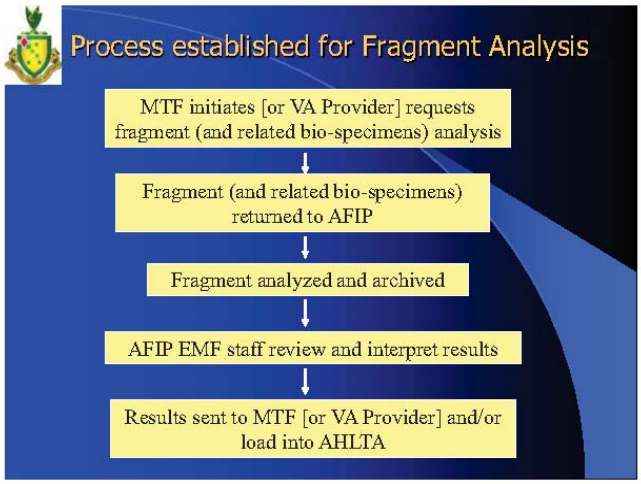

Alternatives to DU
(Tungsten alloys)
Tungsten Uses
Tungsten carbide (WC) and tungsten carbide with cobalt (WC/Co)
Medical implants (artificial joints, rods, stents, wires, etc.)
Hunting ammunition – "Non-toxic shot"
Military uses (W-alloys):
Tungsten + Nickel + Copper (W:Ni:Co)
Tungsten + Nickel + Iron (W:NI:Fe)
Tungsten + Nickel + Iron + Cobalt (W:Ni:Fe:Co)
For special applications
Tungsten + Nickel + Cobalt
Tungsten + molybdenum + Nickel + Iron
(W content > 90 w%; density > 17 g/cm³)

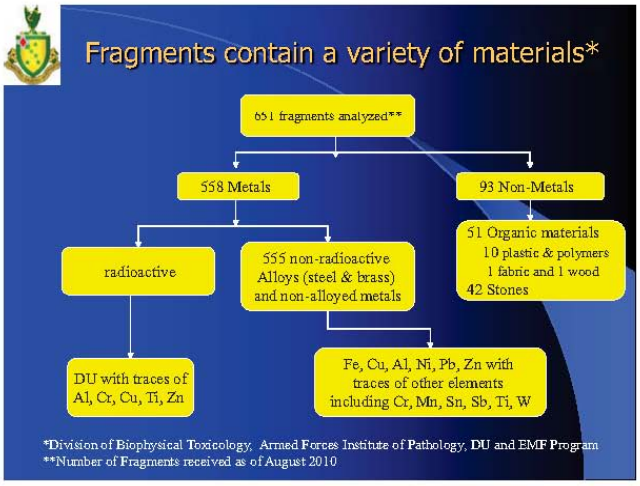

Tungsten Alloy-Associated Tumors*
(Specie: Rats)
Tantalum (control)
Tungsten Alloy
Pellet
Pellet
No tumors in Ta controls
Rapidly growing tumors up to 5 cm in diameter
Tumor surrounds pellet
Tumor displaces and replaces skeletal muscle around pellet
Diagnosis: Rhabdomyosarcoma
Gross photos and photomicrograph: Courtesy of Dr. John F. Kalinich, AFRRI-USUHS
*Kalinich JF, Emond CA, Dalton TK, Mog SR, Coleman GD, Kordell JE, Miller AC, McClain DE. Embedded weapons-grade tungsten alloy shrapnel rapidly induces metastatic high-grade rhabdomyosarcomas in F344 rats. Environ Health Perspect. 2005 Jun;113(6):729-34.

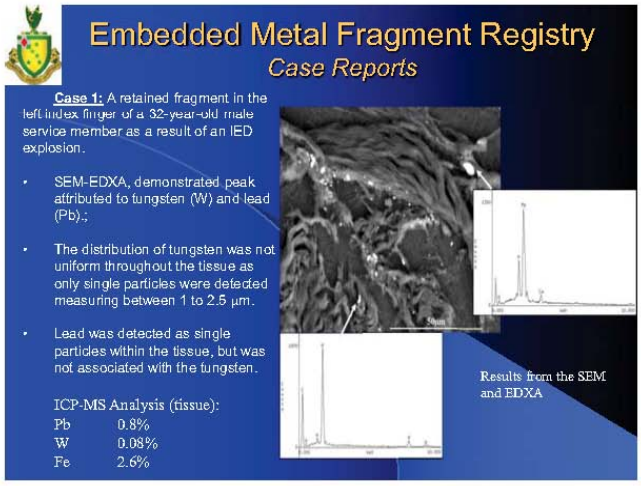

AFIP
What is being done for those concerned about DU and embedded fragment exposures?
Photos: Courtesy of Associated Press


AFIP Depleted Uranium (DU) and Embedded Metal (EMF) Fragment Program
Analyzing fragment composition is an essential function of the AFIP DU and EMF Program
Chemical analysis of removed fragments
Surface chemistry
Total fragment composition (over 35 metals)
Analysis of tissue surrounding fragments
Chemical analysis of tissue
Characterization of tissue morphology
Histology – proliferative cells, neoplastic cells
Analysis of wound and body fluids (whole blood, plasma, urine, etc)
Laser ablation ICP-MS
X-Ray fluorescence spectrometry
Instrumentation and methodology available: ICP OES, ICP MS (quadropole), HR ICP MS, Microscopy (IR and laser based), SEM EDXA, X ray fluorescence


AFIP Depleted Uranium (DU) and Embedded Metal Fragment (EMF) Program
Toxicants of interest
Metals: Al, As, Cd, Cr, Co, Cu, Fe, Mn, Ni, Pb, Sn, Sb, Ti, U, W, Zn
Plastics/polymer components: Isocyanate, Acrylics, Diethylhexylphthalates
Others: Based on fragment analysis data
Biological Specimens
Urine, blood, other body fluids when indicated
Copper-based fragment
Fragments of different sizes and metal compositions


Process established for Fragment Analysis
MTF initiates [or VA Provider] requests fragment (and related bio-specimens) analysis
Fragment (and related bio-specimens) returned to AFIP
Fragment analyzed and archived
AFIP EMF staff review and interpret results
Results sent to MTF [or VA Provider] and/or load into AHLTA

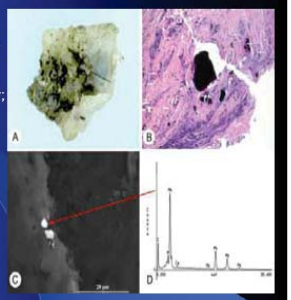

Fragments contain a variety of materials*
651 fragments analyzed**
558 Metals
93 Non-Metals
radioactive
555 non-radioactive Alloys (steel & brass) and non-alloyed metals
51 Organic materials
10 plastic & polymers
1 fabric and 1 wood
42 Stones
DU with traces of Al, Cr, Cu, Ti, Zn
Fe, Cu, Al, Ni, Pb, Zn with traces of other elements including Cr, Mn, Sn, Sb, Ti, W
*Division of Biophysical Toxicology, Armed Forces Institute of Pathology, DU and EMF Program
**Number of Fragments received as of August 2010


Embedded Metal Fragment Registry
Case Reports
Case 1: A retained fragment in the left index finger of a 32-year-old male service member as a result of an IED explosion.
SEM-EDXA, demonstrated peak attributed to tungsten (W) and lead (Pb).;
The distribution of tungsten was not uniform throughout the tissue as only single particles were detected measuring between 1 to 2.5 µm.
Lead was detected as single particles within the tissue, but was not associated with the tungsten.
ICP-MS Analysis (tissue):
Pb 0.8%
W 0.08%
Fe 2.6%
Results from the SEM and EDXA

Embedded Metal Fragment Registry
Case Reports

Case 2: A 25-year-old male with a history of fragment injury to the back of the head and shoulder from a gunshot wound, suffered in 2007 while serving in Iraq.

- (A) Retained fragments in soft tissue for ~1 year;
- (B,C, &D) By SEM-EDXA, black scattered fragments were identified as lead (Pb);
- Quantitative analysis of the tissue by ICP-MS demonstrated a level of 1.1% Pb;
- The absence of other metals on both the SEM-EDXA and ICP-MS analyses indicate that this embedded fragment is made of metallic lead.



Photos and photomicrographs: Jose A. Centeno, AFIP

AFIP Depleted Uranium (DU) and Embedded Metal Fragment (EMF) Program

- Information captured through the analysis of these fragments could be used to:
  - Develop medical and surgical management guidelines for service members and veterans with embedded fragments
  - Track individuals throughout the process
  - Conduct population surveillance

AFIP Depleted Uranium (DU) and Embedded Metal Fragment (EMF) Program

Fragment data informs biomonitoring and medical surveillance protocol

- Fragment composition data helps:
  - Improve clinical management decisions;
  - Identify a list of toxicants to include in biomonitoring panels;
  - Identify outcomes of concern and means of surveillance;
  - Identify potential biomarkers of early effect.

The AFIP EMF registry will help the **DoD (and theVA)** refine how cases are identified and provide appropriate medical care for individuals with embedded fragments.

Health Risks from Retained Metal Fragments
Research Partners and Relevant Programs

I. DU Related - Baltimore VA Medical Center
   a. DU Clinical Follow-up Program
   b. DU Biosurveillance Program

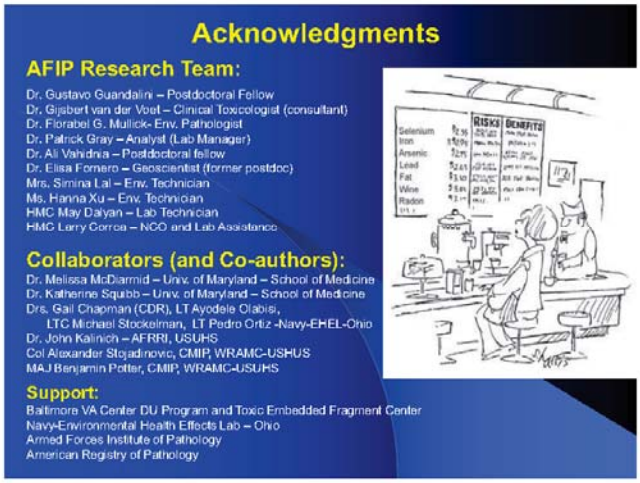
II. VA Toxic Embedded Fragment Center (TEFC)

Point-of-contact: Dr. Melissa McDiarmid, Director, VA Baltimore Medical Center
Email: Mmcdiarm@medicine.umaryland.edu

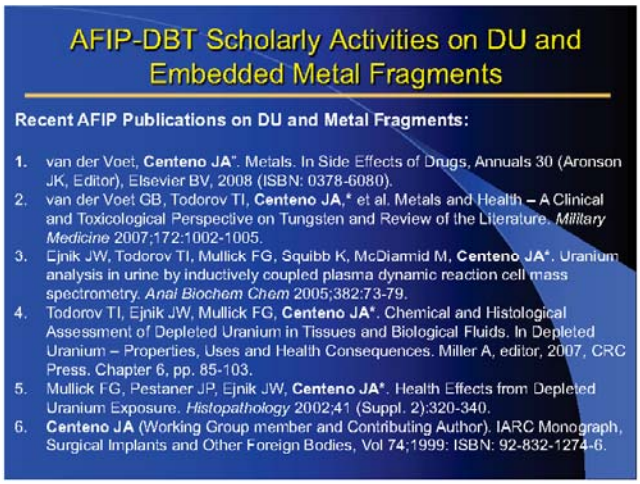
III. WRAMC Combat Wound Initiative Program

Point-of-contact: COL Alexander Stojadinovic, PI, WRAMC-USUHS
Email: Alexander.Stojadinovic@us.army.mil

IV. Toxicology Program – Armed Forces Radiobiology Research Institute
Point-of-contact: Dr. John Kalinich, AFRRI-USUHS
Email: John.Kalinich@us.army.mil


Acknowledgments
AFIP Research Team:
Dr. Gustavo Guandalini – Postdoctoral Fellow
Dr. Gijsbert van der Voet – Clinical Toxicologist (consultant)
Dr. Florabel G. Mullick- Env. Pathologist
Dr. Patrick Gray – Analyst (Lab Manager)
Dr. Ali Vahidnia – Postdoctoral fellow
Dr. Elisa Fornero – Geoscientist (former postdoc)
Mrs. Simina Lal – Env. Technician
Ms. Hanna Xu – Env. Technician
HMC May Dalyan – Lab Technician
HMC Larry Correa – NCO and Lab Assistance
Collaborators (and Co-authors):
Dr. Melissa McDiarmid – Univ. of Maryland – School of Medicine
Dr. Katherine Squibb – Univ. of Maryland – School of Medicine
Drs. Gail Chapman (CDR), LT Ayodele Olabisi,
LTC Michael Stockelman, LT Pedro Ortiz -Navy-EHEL-Ohio
Dr. John Kalinich – AFRRI, USUHS
Col Alexander Stojadinovic, CMIP, WRAMC-USHUS
MAJ Benjamin Potter, CMIP, WRAMC-USUHS
Support:
Baltimore VA Center DU Program and Toxic Embedded Fragment Center
Navy-Environmental Health Effects Lab – Ohio
Armed Forces Institute of Pathology
American Registry of Pathology
RISKS
BENEFITS
Selenium
Iron
Arsenic
Lead
Fat
Wine
Radon


Thank you for your attention!


AFIP-DBT Scholarly Activities on DU and Embedded Metal Fragments
Recent AFIP Publications on DU and Metal Fragments:
1. van der Voet, **Centeno JA***. Metals. In Side Effects of Drugs, Annuals 30 (Aronson JK, Editor), Elsevier BV, 2008 (ISBN: 0378-6080).
2. van der Voet GB, Todorov TI, **Centeno JA**,* et al. Metals and Health – A Clinical and Toxicological Perspective on Tungsten and Review of the Literature. *Military Medicine* 2007;172:1002-1005.
3. Ejnik JW, Todorov TI, Mullick FG, Squibb K, McDiarmid M, **Centeno JA***. Uranium analysis in urine by inductively coupled plasma dynamic reaction cell mass spectrometry. *Anal Biochem Chem* 2005;382:73-79.
4. Todorov TI, Ejnik JW, Mullick FG, **Centeno JA***. Chemical and Histological Assessment of Depleted Uranium in Tissues and Biological Fluids. In Depleted Uranium – Properties, Uses and Health Consequences. Miller A, editor, 2007, CRC Press. Chapter 6, pp. 85-103.
5. Mullick FG, Pestaner JP, Ejnik JW, **Centeno JA***. Health Effects from Depleted Uranium Exposure. *Histopathology* 2002;41 (Suppl. 2):320-340.
6. **Centeno JA** (Working Group member and Contributing Author). IARC Monograph, Surgical Implants and Other Foreign Bodies, Vol 74;1999: ISBN: 92-832-1274-6.

The Evaluation of Nanoparticles as Biological Decontaminants

United States Air Force School of Aerospace Medicine (USAFSAM)/PHT, Brooks City Base, TX

Clarise R. Starr, PhD, George F. Viale, MSgt, USAF, NCOIC, Linda S. Armstrong, MS, Manuel Y. Caballero, BS, and David L. Maserang, PhD

Nanoparticles are insoluble particles that are no greater than 100 nm in size and are reported to have various electronic, magnetic and optical properties associated with them. Recent studies have demonstrated the unique anti-microbial and toxin neutralizing potential of these particles. The objective of this study is to find a universal nanoparticle formulation that is capable of killing Gram positive, Gram negative and spore forming bacteria and viruses, thus providing a potential alternative as a decontaminating solution in hospitals and laboratories, and have the ability to neutralize two common biothreat toxins, C. botulinum A and Staphylococcus enterotoxin B. This research has 3 specific aims: 1) To test a series of commercially based nanoparticle solutions (AgO, MgO and ZnO) with and without halogenation in liquid, powder, and gel matrices in order to find the top 4 solutions that can work on all 4 microorganisms and 2 toxins. 2) To test the top 4 formulations against various clinical and environmental matrices and surfaces to determine strengths and limitations and 3) To further test the safety of these formulation against established assays for possible deleterious effects against the end-user, if any, by established cell culture toxicity studies. This study utilizes commercially available nanoparticle reagents that could be modified to work effectively as a universal decontaminant solution that is safer and less caustic than most off-the-shelf alternatives, such as bleach and in the case of anthrax decontamination, chlorine dioxide gas. The ideal solution would be easy to transport, safe for the end-user to manipulate with minimal protective gear, and effective against bacteria, viruses, and toxins in a short contact time.

U.S. AIR FORCE

The Evaluation of Nanoparticles as Biological Decontaminants

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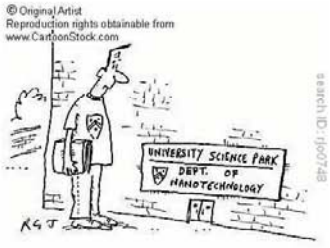
Molecular Biologist
USAFSAM/PHT
Brooks City-Base, TX

Every Airman a Force Multiplier
August 2010 AFMS Research Symposium

Nanotechnology

Every Airman a Force Multiplier



Uses of Nanomaterials

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Everyday Uses	"Biological" Uses
Eyeglasses	Targeted drug delivery
Crack-resistant paints	Targeted gene therapy
Transparent sunscreens	Antimicrobial coatings
Stain-repellent fabrics	Fluorescent labeling
Self-cleaning windows	Imaging contrast
Coatings for solar cells	Tissue engineering
Automotive	DNA probes
Planes	Microsurgical techniques

Nanoparticles and Biocidal Properties

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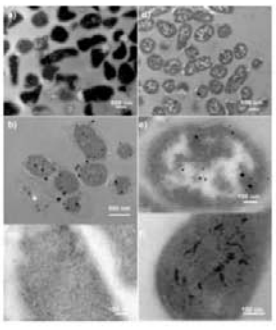


Table 2. Surviving Bacteria Colonies vs mg of Silver Product, Based on 10 min of Contact between Silver and Bacteria in Water[a]

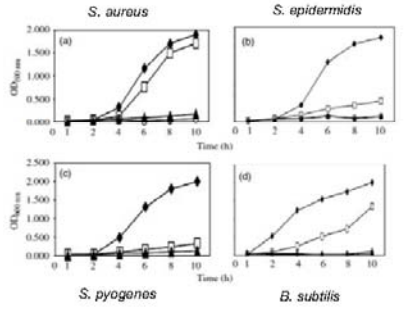
sample	amount of silver sample (mg)	*E. coli* CFUs	*S. aureus* CFUs
no silver (control)	–	100 000	100 000
Powders			
Aldrich 60 mesh	15	79	53
Aldrich 60 mesh	1.5	0	72
Aldrich nano-sized powder	15	0	31
Aldrich nano-sized powder	1.5	0	84
$AgNO_3$	1.5	0	0
$AgNO_3$	0.15	0	0
$AgNO_3$	0.015	0	0
SMAD	15	0	0
SMAD	1.5	–	1
SMAD	0.15	–	53
air–SMAD	0.75	1	2
air–SMAD	0.15	1	0
KOH–SMAD–isopropanol	15	–	0
KOH–SMAD–isopropanol	1.5	7	153
Ligand-Capped Particles			
3-mercapto-1,2,propanediol	15	–	5000
3-mercapto-propanesulfonate	15	175	11
5-mercapto-tetrazole/acetate	15	84	5000
pure ligand (no silver)	0.1 sol.	100 000	100 000
ligand plus SMAD powder	15	10 000	1000
ligand plus $AgNO_3$	1.5	500	100

[a] All values are an average of CFUs taken in triplicate and were reproducible within roughly 5%. All samples were dispersed in water, except $AgNO_3$, which was dissolved in water.

Smetana et al. 2008. Langmuir 24:7457-7464

ZnO as a Decontaminant

Every Airman a Force Multiplier



Jones et. al 2008. FEMS Microbiol Lett 279:71-76

Halogenated Nanoparticles

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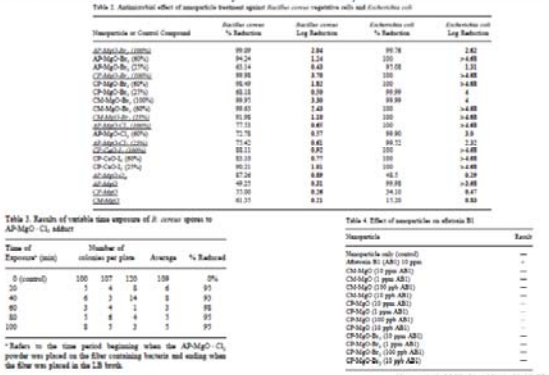

Koper et al. 2002. Curr Microbiol 44:49-55

Goals of the Study

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- Examine commercially available nanoparticle reagents that could be modified to work effectively as a universal decontaminant solution.
- The ideal solution would be easy to transport; safe for the end-user to manipulate with minimal protective gear; and effective against bacteria, viruses, and toxins in a short contact time.
- Solution must be *safer* and less caustic than most off-the-shelf alternatives, such as bleach and, in the case of Anthrax decontamination, chlorine dioxide gas.

Short List of Nanoparticles Used in Study

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Nanoparticle	Fold Decrease in Comparison to Untreated Controls (at 30-minute exposure)			
	Bacillus globigii Spores	*Erwinia herbicola*	*Staphylococcus aureus*	MS2 Bacteriophage
Manganese oxide nanoparticle (Mn_2O_3), 40-60 nm	#	1.93	-	#
Reactive magnesium oxide, PM-40-25	#	#	Complete kill	#
Chlorinated nanoactive magnesium oxide plus suspension	#	#	1.5	#
Brominated nanoactive magnesium oxide plus suspension	#	#	1#	Complete kill
Brominated nanoactive magnesium oxide plus	#	#	2#	#
Iodinated nanoactive magnesium oxide plus suspension	#	#	1.7	2#
Copper oxide nanoparticle (CuO), 40 nm, 99+%	1.4	12.2	-	#
Silver nanoparticle, 100 nm, 99.5%	2.7	#	9	#
Silver nanoparticle, TAT control, 20-30 nm, 99.99%	-	Complete kill*	Complete kill*	#
Silver, 50-80 nm, 99.9%	1.6	#	2#	#
Silver doped nanoactive aluminum oxide plus	-	Complete kill*	4#	#
Aluminum oxide (gamma), >99.8%, AFS 60 nm	-	3.3	-	#
Titanium oxide (brookite phase), >98%, AFS 5-10 nm	#	2	1#	#
Titanium carbide (beta), >99%, AFS <30 nm	2#	Complete kill*	-	#
Titanium nitride, >98%, AFS <15 nm	#	1.3	2	#
Zinc oxide, >99.5%, AFS 20 nm	2#	924	3.4	#
Chlorinated nanoactive zinc oxide suspension	#	#	-	#
Nanoactive zinc oxide, PM-117	1#	6.3	-	#
Brominated nanoactive zinc oxide suspension	-	6.6	-	#
Iodinated nanoactive zinc oxide suspension	#	Complete kill*	Complete kill*	Complete kill

*Effect occurred at all incubation time points.

No observable growth at any incubation time points by 24 hours. At 48 hours, growth at 5 minute exposure was about 25% of untreated control.
Minimal growth also seen at other exposure times by 48 hours, including 1 colony seen for 20 minute exposure time.

SEM of Nanoparticles Used in Study

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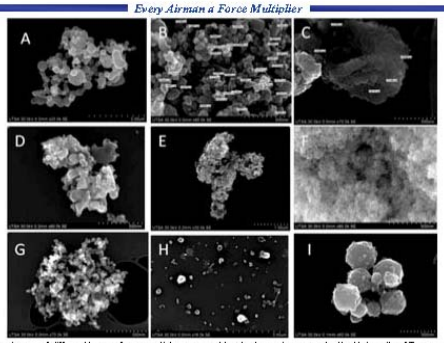

A representative image of different types of nanoparticles scanned by electron microscopy by the University of Texas at San Antonio Department of Physics. **A**. Silver nanoparticle, 100 nm. B. Titanium carbide (beta), APS <30 nm. **C**. Zinc oxide APS 20 nm. D. Silver nanoparticle, PVP coated 20-30 nm. E. Silver nanoparticle, 50-60 nm. F. Aluminum oxide (gamma), APS 60 nm. **G**. Titanium nitride, APS <15 nm. H. Manganese oxide, 40-60 nm. I. Zinc powder, APS 35 nm.

Methods

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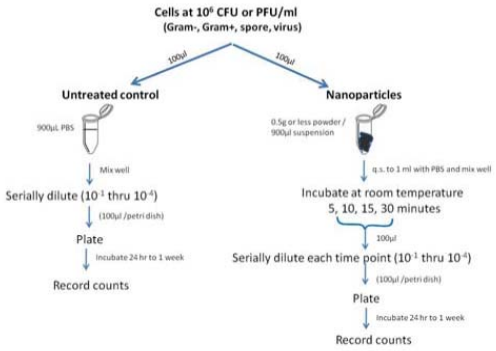

Staphylococcus aureus +/- Nanoparticles

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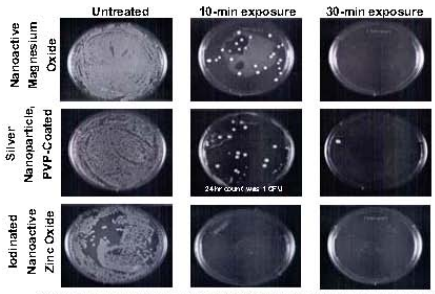

Cultures at a concentration of 9 x 10^5 CFU/ml were exposed to nanoparticles, serially diluted, and then plated. 10^{-1} dilutions shown here. Photos taken after 4 days in culture.

Bacillus globigii +/- PVP-Coated Silver Nanoparticle

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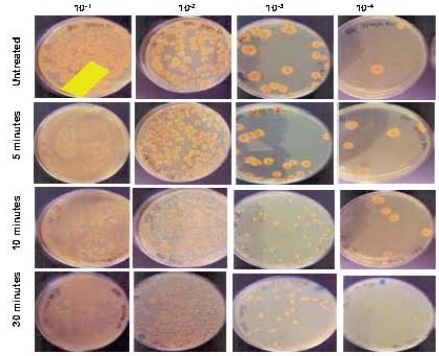

Bacillus globigii +/- PVP-coated Silver Nanoparticle

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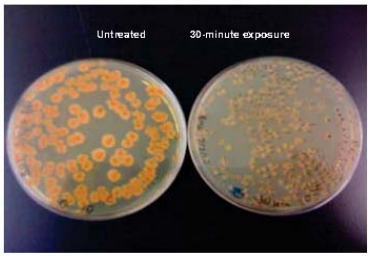

Cultures above were exposed to 750 mg/ml of a PVP-coated silver nanoparticle for 1, 5, 10, and 30 minutes, serially diluted, and plated. The 10^{-2} dilution of the 30-minute exposure is shown here. Variation in size of treated versus untreated also occurred at the 10-minute exposure (except at highest dilution).

Toxin Study with Nanoparticles Using Meso Scale Assay Plates

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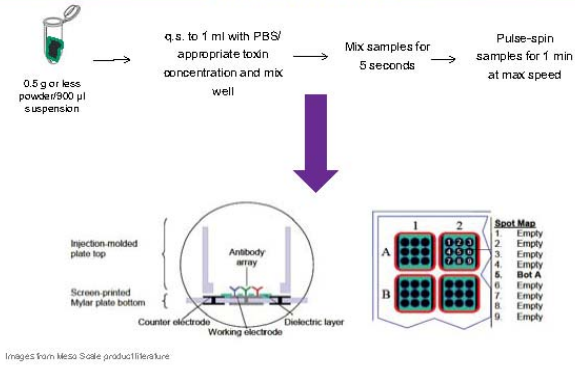

Images from Meso Scale product literature

Ag PVP Binds to CBot A

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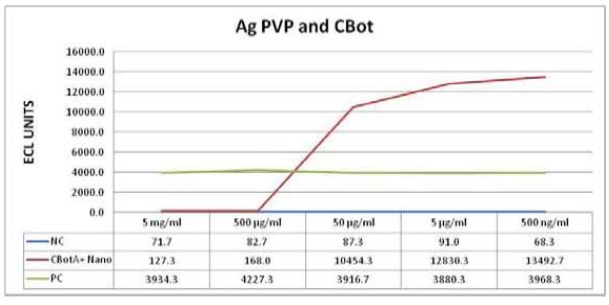

	5 mg/ml	500 µg/ml	50 µg/ml	5 µg/ml	500 ng/ml
NC	71.7	82.7	87.3	91.0	68.3
CBotA+ Nano	127.3	168.0	10454.3	12830.3	13492.7
PC	3934.3	4227.3	3916.7	3880.3	3968.3

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Conclusions

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- Several of the nanoparticles tested had a bacteriostatic effect rather than a bactericidal effect on the 4 microorganisms tested.
- No real bactericidal effect has been observed on spores---this may be due to the assay conditions (suspension versus dry), and steps to modify assay are taking place.
- Toxin neutralization preliminary data have shown a decrease in toxin signal using the MSD 1800 CBot assay. It is currently unknown if nanoparticle binding is specific and permanent.
- Ag (PVP)-coated, iodinated nanoactive Zn, silver-doped nanoactive AlO plus, chlorinated nanoactive MgO plus suspension, and brominated nanoactive MgO plus suspension are good potential candidates for toxicity study.

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Jose Yacaman, Ph.D.

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Toxicology & ESOH Issues of Engineered Nanomaterials

711th Human Performance Wing (HPW)/RHPA

Saber Hussain, Laura Braydich-Stolle, Nicole Schaeublin, David Mattie

(Presented by Dr. David Mattie)

Recent developments have generated a degree of apprehension concerning potential environmental, safety and occupational health (ESOH) risks associated with new, engineered nanomaterials. We are conducting focused research to establish the possible effects of nanoparticle exposure on biological systems. There are a great variety of physiochemical properties such as size, shape and surface chemistry of nanoparticles, which can contribute to nanotoxicity and this makes the safety assessment a challenging problem. We have established a lung co-culture model that simulates the human lung environment to evaluate the respiratory toxicity of nanoenergetic materials. We have demonstrated that there is a size dependent toxic effect of silver and silica nanoparticles, while in terms of gold nanotoxicity size, charge, and shape were mediating factors. When keratinocytes were exposed to gold nanospheres and rods, the rod shaped gold induced more toxicity. Furthermore, charged gold nanoparticles induced apoptosis, while neutral gold nanoparticles did not. Additionally, studies with nanoenergetic aluminum have demonstrated that at low levels of exposure there was little toxicity in the lung co-cultures, however, the immune cells ability to respond to bacterial pathogens was reduced. Taken together, all of these nanotoxicity studies demonstrate that there are multiple parameters that will contribute to how nanomaterials interact with a biological system and it is imperative to characterize these materials in order to fully understand the biological responses. The main focus of this presentation will be to discuss basic research applied to discover biological interaction of nanomaterials and its relationship to potential human health concerns.

AFRL
THE AIR FORCE RESEARCH LABORATORY
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Toxicology & ESOH Issues of Engineered Nanomaterials

Aug 25, 2010

Saber Hussain, PhD
Nicole Schaeublin, MS
Laura K. Braydich-Stolle, PhD
Dave Mattie, PhD
Human Effectiveness Directorate
Air Force Research Laboratory
Wright-Patterson AFB, OH 45433

Project Goals & Objectives

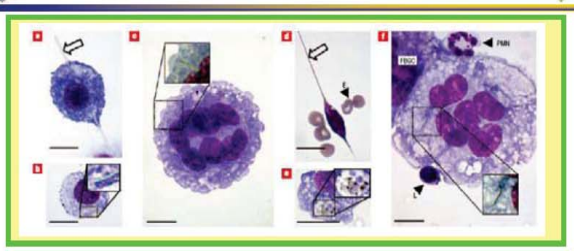
- Establish tools to evaluate toxicity to address ESOH issues of NM
  - Establish relationship between toxicity and size, morphology, and composition of engineered nanomaterials
  - Address tech challenges for assessing uptake, translocation and management of nanomaterials in a live cell environment
  - Identify exposure concentrations of nanomaterials that produce adverse effects in cells and organs at doses which are representative of occupational exposure
- Establish fundamental ground work to investigate effects & mechanism(s) of biological interaction of NM
  - Explore concept(s) of using NM as intracellular sensors to modulate biological function

Why ESOH is Necessary?: DoD Relevance

Nano ESOH policy letter, signed by the Under Secretary of Defense, reinforces responsibilities for managing ESOH risks of engineered nanomaterials in DoD research laboratories.

Recent Reports in Literature

Carbon Nanotubes Shows Asbestos-like Pathogenicity in a Pilot Study- Mouse Model



- Carbon nanotubes – characterized by their microscopic size and incredible tensile strength – similarities to asbestos, due to their needle-like fiber shape.
- In a recent study that introduced carbon nanotubes into the abdominal cavity of mice, results demonstrated that long thin carbon nanotubes showed the same effects as long thin asbestos fibers, raising concerns that exposure to carbon nanotubes may lead to mesothelioma (cancer of the lining of the lungs caused by exposure to asbestos)

Poland et al Nature Nanotechnology 3, 423 - 428 (2008)

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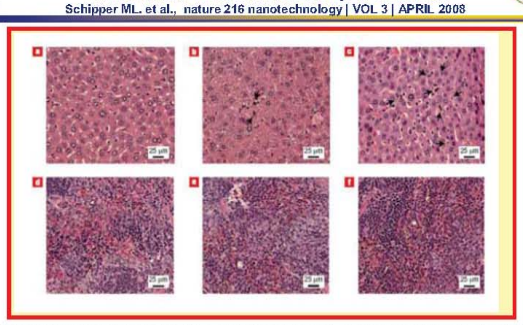
A pilot toxicology study of single-walled carbon nanotubes in a small sample of mice

Schipper ML. et al., nature 216 nanotechnology | VOL 3 | APRIL 2008



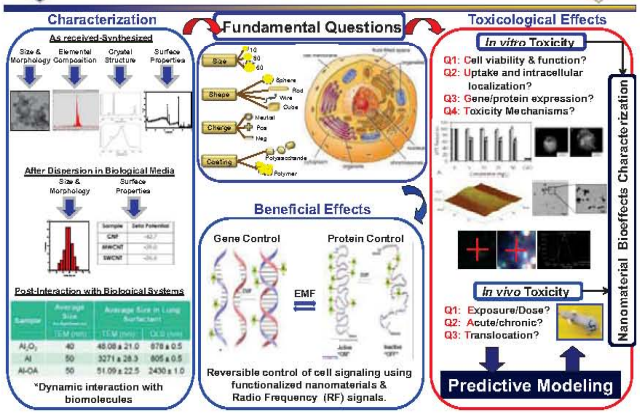
- Figure 3 Liver and spleen histology. a–f, Haematoxylin and eosin stains of liver (a–c) and spleen (d–f) tissues of mice injected with PBS (a,d), SWNT PEG (b,e) or SWNT O PEG (c,f). Finely granular brown-black pigments were seen in sinusoidal liver cells of SWNT PEG (b, arrows) and SWNT O PEG (c, arrows), as well as a golden-brown pigment in spleen macrophages of SWNT PEG and SWNT O PEG (e,f), without signs of cellular or tissue damage

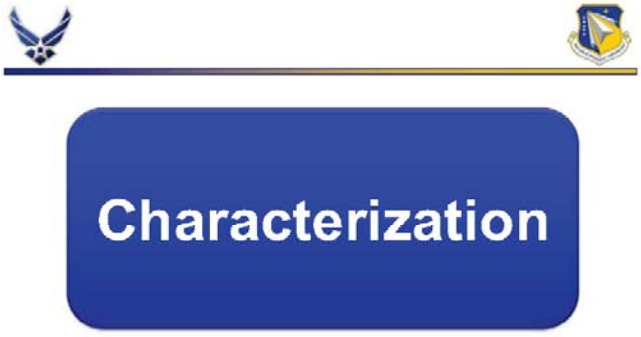
6

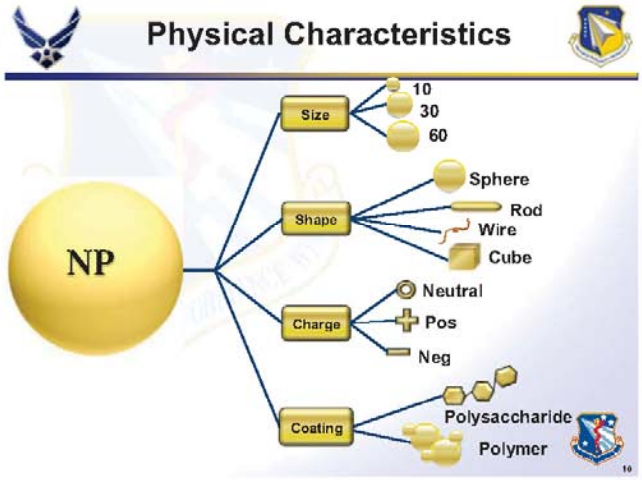
OVERVIEW
Project Approach

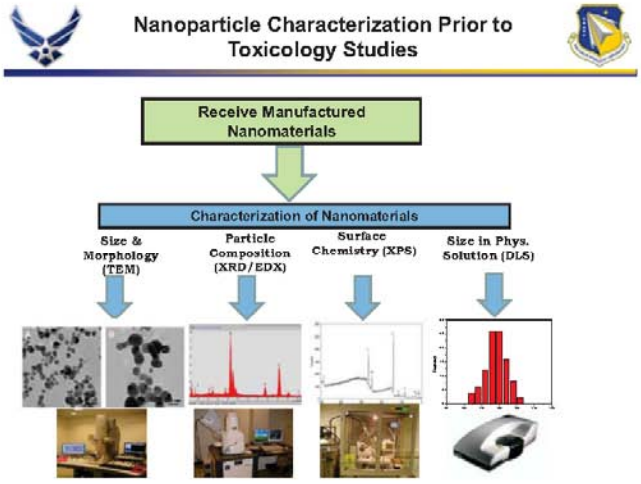
7

Biological Interaction of Nanomaterials

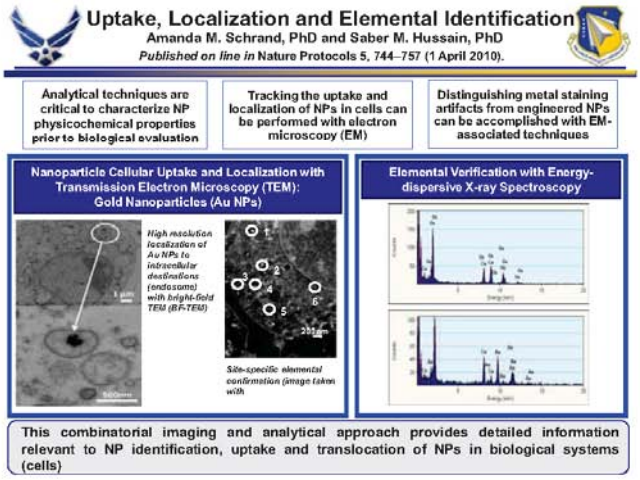


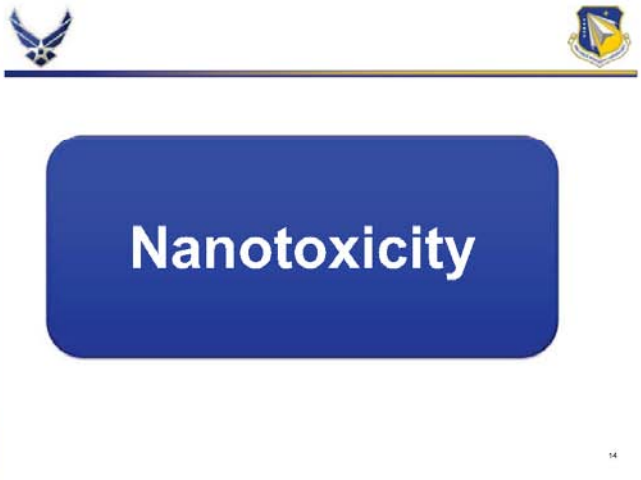

Characterization

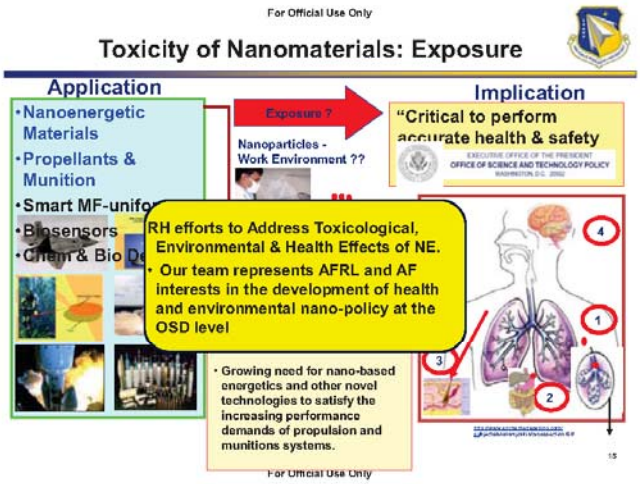

Physical Characteristics
NP
Size
10
30
60
Shape
Sphere
Rod
Wire
Cube
Charge
Neutral
Pos
Neg
Coating
Polysaccharide
Polymer

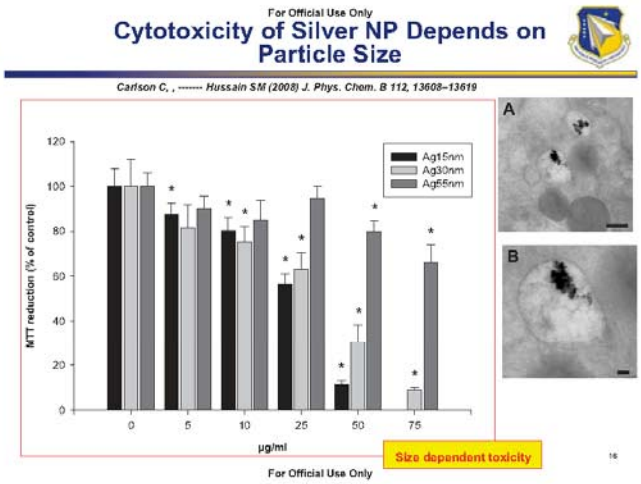

Nanoparticle Characterization Prior to Toxicology Studies
Receive Manufactured Nanomaterials
Characterization of Nanomaterials
Size & Morphology (TEM)
Particle Composition (XRD/EDX)
Surface Chemistry (XPS)
Size in Phys. Solution (DLS)

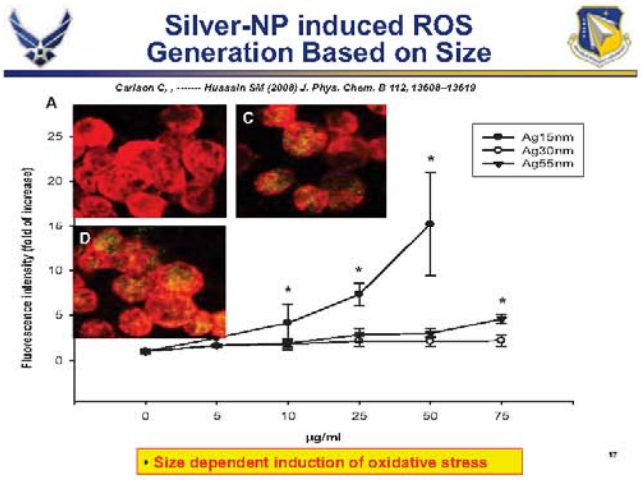

Editorial Highlight in Toxicological Sciences
IMPACT
TOXICOLOGICAL HIGHLIGHT
How Meaningful are the Results of Nanotoxicity Studies in the Absence of Adequate Material Characterization?
Editor
Murdock RC, Braydich-Stolle L, Schrand AM, Schlager JJ and Hussain SM (2007) Characterization of nanomaterial dispersion in solution prior to in vitro exposure using dynamic light scattering technique. Toxicol Sci 101:239-253.

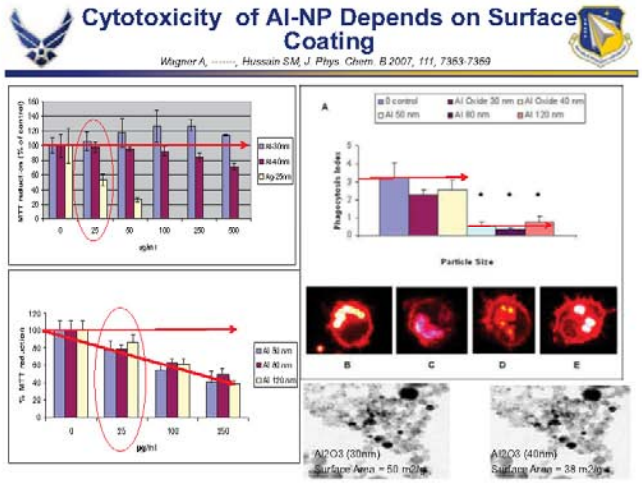

Uptake, Localization and Elemental Identification
Amanda M. Schrand, PhD and Saber M. Hussain, PhD
Published on line in Nature Protocols 5, 744–757 (1 April 2010).
Analytical techniques are critical to characterize NP physicochemical properties prior to biological evaluation
Tracking the uptake and localization of NPs in cells can be performed with electron microscopy (EM)
Distinguishing metal staining artifacts from engineered NPs can be accomplished with EM-associated techniques
Nanoparticle Cellular Uptake and Localization with Transmission Electron Microscopy (TEM): Gold Nanoparticles (Au NPs)
Elemental Verification with Energy-dispersive X-ray Spectroscopy
This combinatorial imaging and analytical approach provides detailed information relevant to NP identification, uptake and translocation of NPs in biological systems (cells)

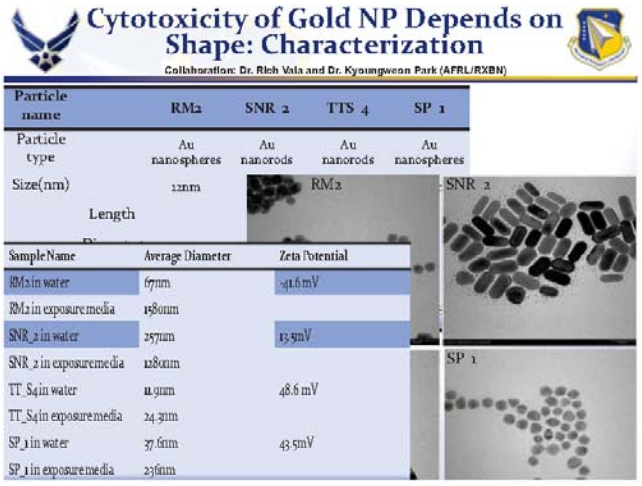

Nanotoxicity

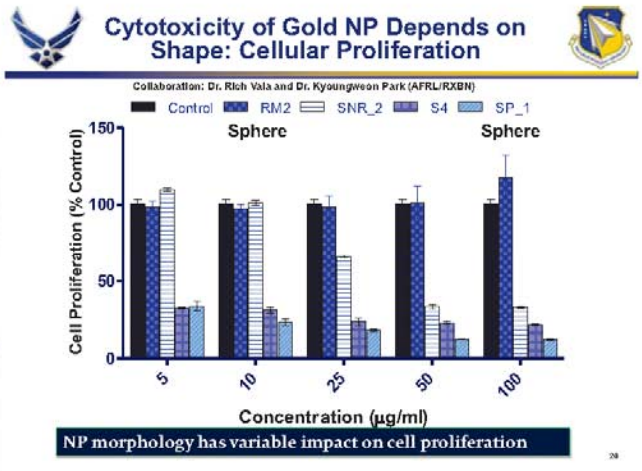

For Official Use Only
Toxicity of Nanomaterials: Exposure
Application
•Nanoenergetic Materials
•Propellants & Munition
•Smart MF-unifo
•Biosensors
•Chem & Bio De
Exposure ?
Nanoparticles - Work Environment ??
Implication
"Critical to perform accurate health & safety
RH efforts to Address Toxicological, Environmental & Health Effects of NE.
• Our team represents AFRL and AF interests in the development of health and environmental nano-policy at the OSD level
• Growing need for nano-based energetics and other novel technologies to satisfy the increasing performance demands of propulsion and munitions systems.
For Official Use Only

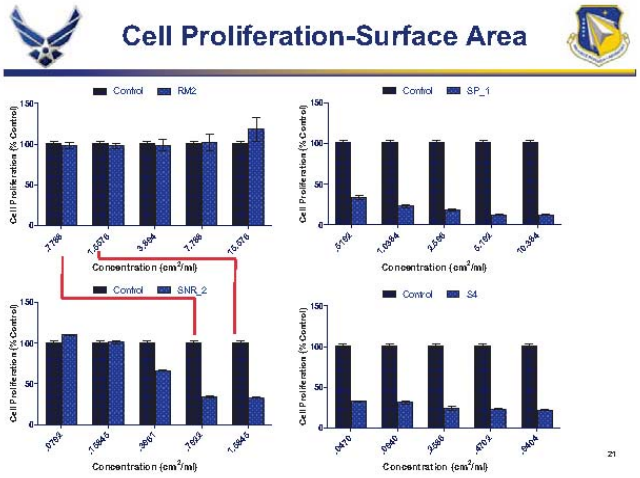

For Official Use Only
Cytotoxicity of Silver NP Depends on Particle Size
Carlson C, , ------- Hussain SM (2008) J. Phys. Chem. B 112, 13608–13619
MTT reduction (% of control)
Ag15nm
Ag30nm
Ag55nm
µg/ml
A
B
Size dependent toxicity
For Official Use Only

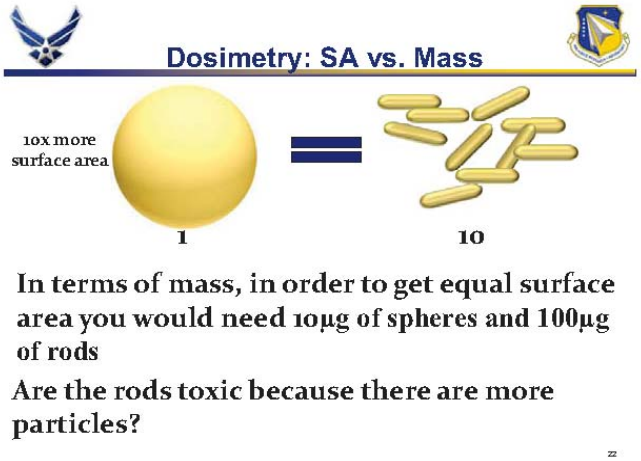

Silver-NP induced ROS Generation Based on Size
Carlson C., ... Hussain SM (2008) J. Phys. Chem. B 112, 13608–13619
A
C
D
Ag15nm
Ag30nm
Ag55nm
Fluorescence intensity (fold of increase)
0
5
10
25
50
75
µg/ml
• Size dependent induction of oxidative stress

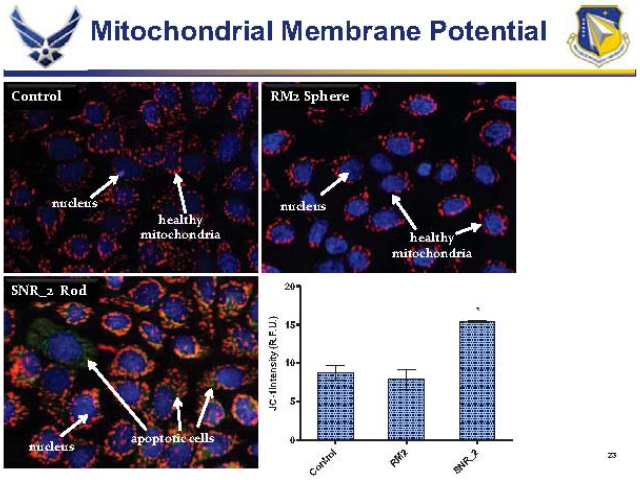

Cytotoxicity of Al-NP Depends on Surface Coating
Wagner A., ... Hussain SM, J. Phys. Chem. B 2007, 111, 7363-7369
Particle Size
Al2O3 (30nm)
Surface Area = 50 m2/g
Al2O3 (40nm)
Surface Area = 38 m2/g

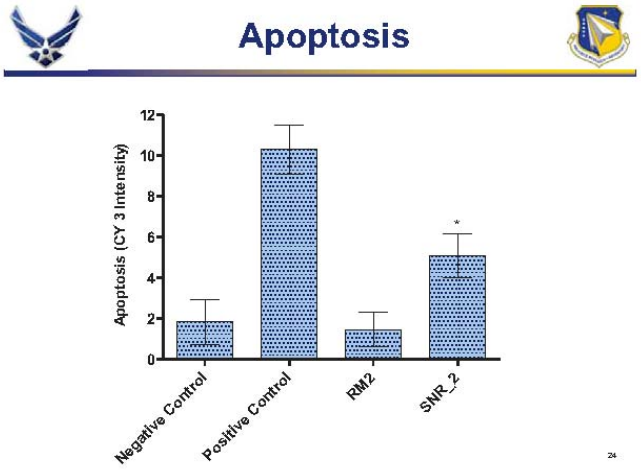

Cytotoxicity of Gold NP Depends on Shape: Characterization
Collaboration: Dr. Rich Vala and Dr. Kyoungweon Park (AFRL/RXBN)
Particle name
RM2
SNR 2
TTS 4
SP 1
Particle type
Au nanospheres
Au nanorods
Au nanorods
Au nanospheres
Size(nm)
12nm
Length
RM2
SNR 2
SP 1
Sample Name
Average Diameter
Zeta Potential
RM2 in water
67nm
-41.6 mV
RM2 in exposure media
158nm
SNR_2 in water
287nm
13.5mV
SNR_2 in exposure media
128nm
TT_S4 in water
11.9nm
48.6 mV
TT_S4 in exposure media
24.3nm
SP_1 in water
37.6nm
43.5mV
SP_1 in exposure media
236nm

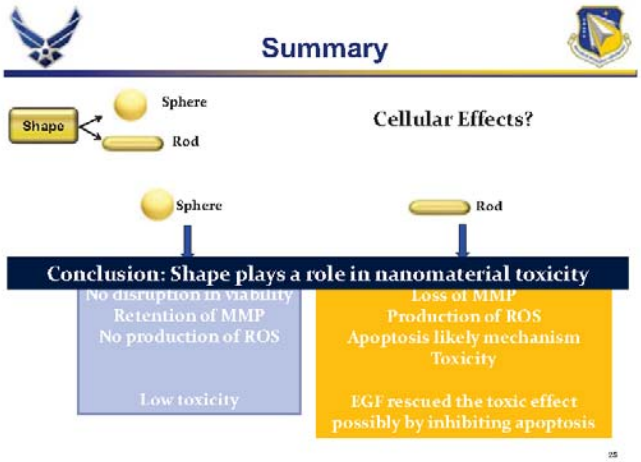

Cytotoxicity of Gold NP Depends on Shape: Cellular Proliferation
Collaboration: Dr. Rich Vala and Dr. Kyoungweon Park (AFRL/RXBN)
Control
RM2
SNR_2
S4
SP_1
Sphere
Sphere
Cell Proliferation (% Control)
150
100
50
0
5
10
25
50
100
Concentration (µg/ml)
NP morphology has variable impact on cell proliferation

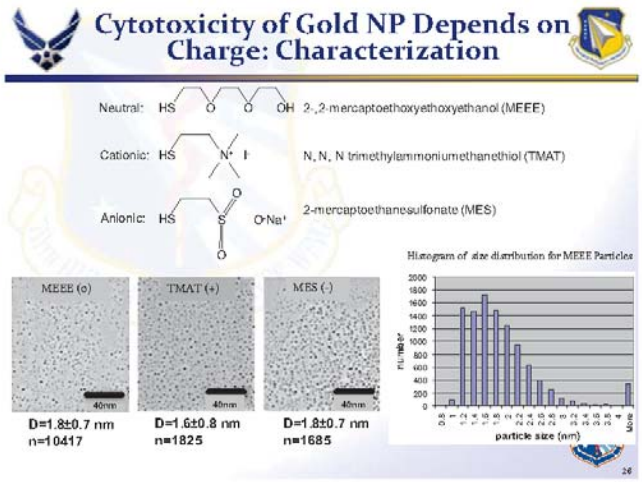

Cell Proliferation-Surface Area
Control
RM2
SP_1
SNR_2
S4
Cell Proliferation (% Control)
Concentration (cm²/ml)
21

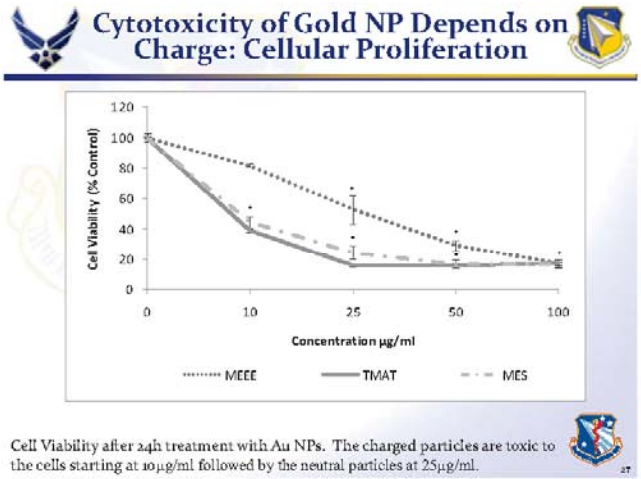

Dosimetry: SA vs. Mass
10x more surface area
1
10
In terms of mass, in order to get equal surface area you would need 10µg of spheres and 100µg of rods
Are the rods toxic because there are more particles?
22


Mitochondrial Membrane Potential
Control
nucleus
healthy mitochondria
RM2 Sphere
nucleus
healthy mitochondria
SNR_2 Rod
nucleus
apoptotic cells
JC-1 Intensity (R.F.U.)
Control
RM2
SNR_2
23

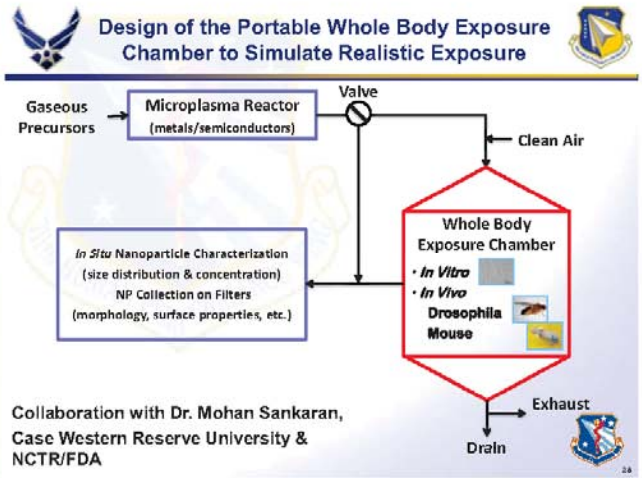

Apoptosis
Apoptosis (CY 3 Intensity)
Negative Control
Positive Control
RM2
SNR_2
24







Cell Viability after 24h treatment with Au NPs. The charged particles are toxic to the cells starting at 10µg/ml followed by the neutral particles at 25µg/ml.



Milestones

- Successfully developed methods to characterize NM in phys environ - Published in Tox Sci
- Successfully established a strong foundation in Bio nano area - Discovered mechanisms of interaction - Published in peer reviewed journals
- Successfully developed methods to determine uptake and translocation of NM in cells - Published in Nature Protocol
- Successfully identified toxicological properties of NM based on size, charge, shape, functionization - Addressed potential toxicological risks of NM - Published in peer reviewed journals
- Establishing a strong foundation that serves as a nexus for multidisciplinary research in the area of Nano-Biotechnology

What's the Message?

- Size matters, but not always
- Physicochemical character matters
  - Size
  - Coating
  - Shape
  - Charge
- Agglomeration vs. dispersion- Critical point
- Charge matters (affects reactivity and dispersion)

Good Nanotoxicology Requires Good Characterization

ESOH SUPPORT

WPAFB/AFRL-Participation in DoD Nano-working Group

Comprehensive Safety Procedure/Protocols

Government Audit (GAO)

Nanotechnology Uses & Risks

1. How would you summarize what is currently known about the hazard or toxicity of manufactured nanomaterials to humans? Are there materials that pose a greater risk than others in terms of hazard? If so, which ones? What about for future products and risks and populations?
2. What is known about the effect that the exposure route (inhalation, dermal absorption, or ingestion) to manufactured nanomaterials has on their toxicity? In what way does the toxicity of certain nanomaterials vary by exposure route?
3. What is known about the extent to which manufactured nanomaterials are toxic or hazardous to the environment? Which manufactured nanomaterials pose the greatest known environmental risks?
4. What effect, if any, does the environmental exposure route (air, water, soil, plant uptake) of manufactured nanomaterials affect the toxicity of nanomaterials to the environment or humans that may encounter them in the environment?

Link

OSD working group

DoD established an inter Service/OSD working group on nanomaterials to coordinate ESOH technical, policy, legal information and actions.

AFRL ESOH Representative:
- Dr. Saber Hussain (RHPB)
- Dr. Richard Vaia, (RXBN) (Advisor)

RHPB/ESOH Nanomaterial Safety Guidelines

This instruction is meant to supplement existing RHPB chemical hygiene plans and incorporates "Approaches to Safe Nanotechnology" recommendations from NIOSH. Link

2nd USAF ASC/AFRL Nanomaterial Workshop

- Held in Fairfield, Ohio at the Holiday Inn on 3-5 November 2009
  - 122 Attendees
  - 29 Speakers
  - Provided a tour at the National Composite Center (NCC)
- Provided Training Credit
  - Continuing Maintenance points for Certified Industrial Hygienist (CIH)
  - Continuing Learning Points (CLP) for Acquisition Professionals Development Program (APDP)

Mr. William LaFountain, ASC/ENVV
Dr. Saber Hussain, AFRL/RHPB

ESOH-SBIR PHASE II

Luna Innovations Incorporated	*Nanocomposix*
• *Web Interfaced Nanotechnology ESOH Guidance System (WINGS™)* – *Air Force SBIR Phase II Contract #FA8650-08-C-6852* – *Dr. Aaron C. Small—*	• *Impact of Nanomaterials on Occupational Safety & Health* – *Air Force SBIR Phase II Contract #FA8650-08-C-6853* – *Dr. Steven Oldenburg-*

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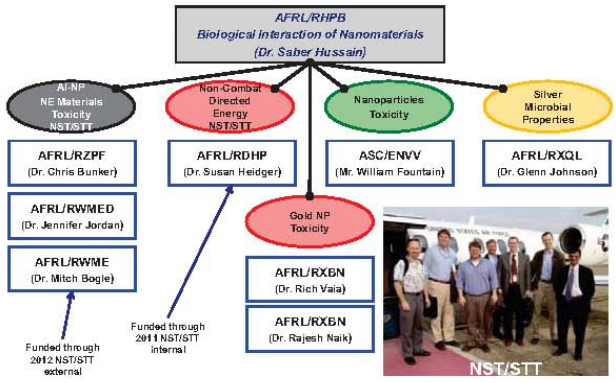
Collaborations

Cross-Directorate Collaborations



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Acknowledgements

RHPB
Col. Reilly
Dr. Schlager
Maj Ramos & Maj Mitchell
The BIN Group Members for team efforts

RXBN
Dr. Rich Vaia
Dr. Kyoungweon Park

Dr. Morley Stone for his support and encouragement

$$$ AF $urgeon General's Office $$$

Thank You!

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QUESTIONS?

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Evaluation of Jet Fuel Inducted Hearing Loss in Rats

711th Human Performance Wing (HPW)/RHPA

David Mattie, PhD

Noise-induced hearing loss (NIHL) continues to be a major military operational problem as well as a general occupational health hazard. Twenty-eight-day studies with male and female rats were designed to study the combined JP-8 jet fuel and noise effects on hearing loss. The first study was a baseline study for creating noise levels similar to occupational exposure. Rats were exposed to 0, 75, 85 or 95 dB for 6 hours per day, 5 days per week over 4 weeks. The second study will be an occupational exposure to noise combined with JP-8 to investigate the combined effects of jet fuel and noise on hearing loss. For noise exposure, audio editing software was used to filter and equalize a white noise file to one octave-band wide, centered at 8 KHz. The signal was split into three equalizers and amplifiers for producing the three noise levels generated using electrodynamic shakers mounted to the exposure chambers. In the first noise-only study, hearing loss was tested by performing the distortion product otoacoustic emission (DPOAE) test used to evaluate hearing function and the compound action potential (CAP) test to determine hearing threshold. Following the hearing assessment, microscopic examination of tissue in the cochlea of the inner ear was conducted to determine the percentage of hair cell receptor loss. All data from the first study showed significant effects on hearing at 95 dB with little or no effects at 75 dB, thus supporting the use of 85 dB for subsequent noise exposures.

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Evaluation of Jet Fuel Induced Hearing Loss in Rats

Air Force Medical Research Symposium
25 August 2010

DAVID R. MATTIE, PhD, DABT
Senior Research Toxicologist
711 HPW/RHPB
Air Force Research Laboratory

Outline

- Goal and Objectives
- Background
- Methods
  - Basic Design
- Study 1
  - Methods
  - Results
- Study 2
  - Methods
  - Results to date
- Future
  - Studies 3 and 4

Goal and Objectives

- Goal
  - To show if there is an association between jet fuel exposure and noise-induced hearing loss
    - The hypothesis is that JP-8 jet fuel contributes to hearing loss when combined with high noise exposure that is still below the exposure limit for noise.
- Objectives
  - Design noise generation system for Navy exposure chambers
  - Conduct four 28-day animal studies
  - Compare jet fuel exposures and kinetics
  - Publication showing relationship between noise and jet fuel exposure

Background

- Dr Fechter exposed rats to 1000 mg/m³ JP-8 for 4 hours followed by either:
  - noise (105 dB) for 4 hours for 1 day
  - noise (97 dB) for 4 hours; repeated for 5 days
  - noise (102 dB) for 1 hour; repeated for 5 days
- Did not examine multiple dose levels of jet fuel
- Noise level equivalent to PEL for noise
  - 90 dB using the "A" weighting scale for 8 h TWA
- JP-8 alone did not cause any disruption of auditory function
- Although effects were not consistent, combined JP-8 + noise produced greater impairment than noise alone
- JP-8 caused significant depletion of GSH, indicating oxidative stress as possible mechanism of action for promotion of hearing loss
- Initial data support interaction between JP-8 and noise exposure on hearing

Fechter, L.D. Gearhart, C., Fulton, S., Campbell, J. Fisher, J., Na, K., Gocker, D., Nelson Miller, A., Moon, P., and B. Pouyatos. (2007). JP-8 Jet Fuel can Promote Auditory Impairment Resulting from Subsequent Noise Exposure in Rats. Toxicol. Sci., 98, 510-525

Background

- Dr Fechter exposed rats in nose-only chambers
- Subsequent study by his lab showed
  - Acute nose-only exposures with clean, filtered air caused significant depletion of liver GSH levels
  - Same effect seen with JP-8
  - Raises questions about combined JP-8 and noise effects seen in Fechter *et al.* (2007)
  - Need whole body exposure chambers

• Fechter, L.D., Nelson-Miller, A. and Gearhart, C. (2008). Depletion of Liver Glutathione Levels in Rats: A Potential Confound of Nose-Only Inhalation. Inhalation Toxicology, 20:905–910

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Methods

- Basic Design
  - Whole body exposure chambers
    - Eliminate stress that may have contributed to previous findings
  - Noise and JP-8 at the same time
  - Three concentrations of JP-8 plus noise level known to produce mild hearing loss
  - Longer time of exposure and more days of exposure

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Methods

- Major accomplishments:
  - Noise system for inhalation chambers developed by
    - MS Graduate Student - John Stubbs, Capt, USAF
    - Under mentorship of Maj Jeremy Slagley – AFIT Assistant Professor
  - Transitioned DPOAE hearing loss measurement system to Navy in collaboration with Dr. Fechter

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Methods – Study 1

Study 1 - Exposure to noise only

- Determined level of noise to use in combined noise and JP-8 study
- Noise exposures at RHPB & EHEL, WPAFB
- Screened rats for normal hearing (DPOAE testing by EHEL)
- Exposed rats for 6 h/day with weekends off for 28 days (20 exposures total)

Group	Exposure Level (db)	Number of Animals	
		Males	Females
Control	0	5	5
Low	75	5	5
Intermediate	85	5	5
High	95	5	5
Total		20	20

- Transported rats to Loma Linda for auditory assessment

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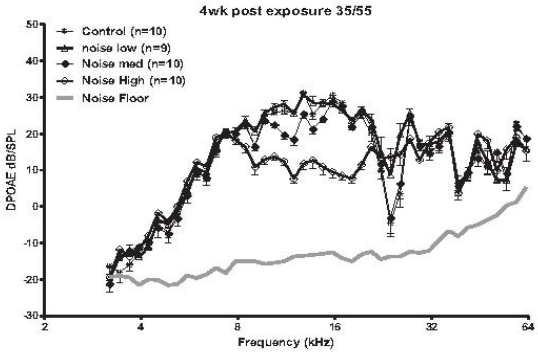
Methods

Hearing loss tested by Distortion Product OtoAcoustic Emission (DPOAE) test

- Test added as screen for normal hearing prior to each 28-day study
  - Assesses hair cell function
  - Transient and permanent impairments as well as recovery rate
- Intact cochlea generates sound energy when stimulated with two simultaneous tones ("primary tones") - frequencies "f1" & "f2"
  - Sound energy generated by cochlea ("distortion products") are different frequencies than "primary tones"
- Detects impairment of hair cells - drop in DPOAE amplitude as function of length along basilar membrane

- Rats lightly anaesthetized with ketamine & xylazine
- f1 & f2 primaries presented through two separate realistic dual radial horn tweeters
- Tones delivered to outer-ear canal through probe - acoustically mixed to avoid artifactual distortion
  - Ear-canal sound pressure levels measured by emissions microphone assembly embedded in probe

Results – Study 1

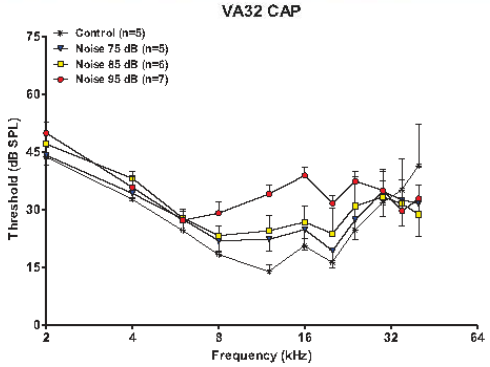


Methods

Hearing loss assessed by Compound Action Potentials (CAPs) test for hearing threshold

- Performed 4 weeks following end of exposures
- Record CAPs from round window for pure tones between 2 & 40 kHz in approximately ½ octave steps
- Auditory thresholds assessed in double walled audiometric booth
- Rats anesthetized (xylazine & ketamine)
- Auditory bulla opened via ventrolateral approach allows placement of fine (od 0.1 mm) Teflon-coated silver wire electrode onto round window
  - Silver chloride reference electrode inserted into neck musculature
- Cochlea warmed using low voltage high-intensity lamp
- CAP signals evoked by pure tones amplified 31000 between 0.1 & 1.0 kHz with Grass A.C. preamplifier
- Identified sound level necessary to generate visually detectable CAP response
  - Averaged over 4 sweeps on digital oscilloscope (approximate response amplitude of 1 mV measured as output of preamplifier)

Results – Study 1



Methods

Hearing loss tested by microscopic examination of inner ear (cochlea)

- Percentage of receptor loss in the ear
  - Outer hair cells (OHCs) & inner hair cells (IHCs)
- Cochleae harvested after CAP measurements
- Cochleae immersed in primary fixative within 2 min
  - After 24 h, tissue washed, postfixed for 2 h, washed again
  - Organ of Corti dissected in 70% ethanol & mounted in glycerin
- Hair cells counted as present when either stereocilia, cuticular plate or cell nucleus could be visualized
  - No attempt to assess degree of possible cellular damage to surviving cells
- Frequency-place "map" established by Muller (1991) superimposes frequency coordinates on length coordinates of organ of Corti
  - Cochlea organized tonotopically
  - High frequency sound produces maximum stimulation of cells in base & low frequency sound in apex

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Methods

Hearing loss tested by microscopic examination of inner ear (cont.)

- Cochleogram shows percentage of hair cell loss as a function of distance from base of cochlea plotted for each animal
- Results averaged across each group of subjects for comparison between groups
- Software used for counting cochlear hair cells developed by R. Lataye and Dr. P. Campo from the "Institut National de Recherche et Se´curite´" (Nancy, France)

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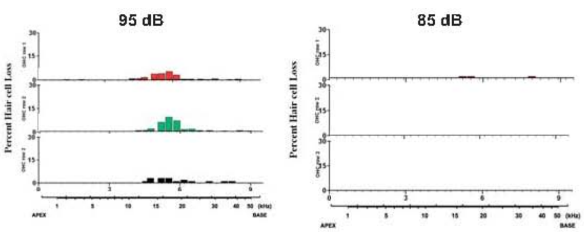
Results – Study 1



75 dB – no hair cell loss

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Methods – Study 2

Study 2 - Exposure to noise & JP-8

- Combined effects of jet fuel & noise on hearing loss.
- Noise + JP-8 exposures in Bldg 837
- Study initiated 28 Apr 10
- 6 h/day with weekends off for 28 days (20 exposures total)
- Transported rats 1 Jun 10 to Loma Linda for auditory assessment

- 16 rats added for Study 2 - collect data for target tissue analysis
  - Lung, blood, liver, fat & brain collected at RHPB
  - Samples transported to analytical lab
  - Compare JP-8 exposure data to previous studies

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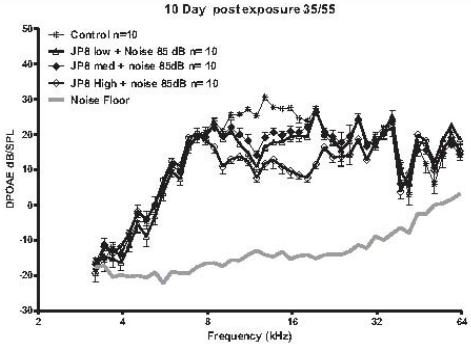
Results – Study 2



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Future

Study 3 - Exposure to JP-8 alone

- Will provide data for comparing effects of jet fuel ototoxicity to noise-induced hearing loss in the combined study
- Study design same as Study 2 without noise
  - 6 h/day with weekends off for 28 days (20 exposures total)
  - JP-8 exposures in Bldg 837
  - Transport rats to Loma Linda for auditory assessment

Study 4 - Exposure to a series of intermittent high levels of noise during JP-8 inhalation exposure

- Will show if high levels of noise for short periods of time during continuous jet fuel exposure can result in hearing loss
- 6 h/day with weekends off for 28 days (20 exposures total)
- Noise + JP-8 exposures in Bldg 837
- Transport rats to Loma Linda for auditory assessment

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Future

Study 4 - Exposure to a series of intermittent high levels of noise during JP-8 inhalation exposure

- Preliminary Design
  - Chamber 1 – air only
  - Chamber 2 – JP-8 only (750 or 1500 mg/m^3)
  - Chamber 3 – noise at 102 dBA for 15 minutes every hour (1.5 h total noise exposure)
  - Chamber 4 - both JP-8 (same as Chamber 2) and noise (same as Chamber 3)

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Acknowledgements

Collaborators

- Dr Jeff Fisher - Co-Principal Investigator
  - University of Georgia, Environmental Health Science
  - Now at NCTR, FDA, AR
- Dr Larry Fechter - Co-investigator
  - Loma Linda VA Medical Center
- LT Vish Mokashi, Ph.D. - Co-investigator
  - Naval Health Research Center Detachment/Environmental Health Effects Laboratory
  - Now at NRL Detachment RDECOM, Edgewood, MD
- CDR Gail Chapman, Ph.D. - Co-investigator
  - Naval Health Research Center Detachment/Environmental Health Effects Laboratory
  - Now at U.S. Army Medical Research & Materiel Command: Navy Liaison Military Infectious Disease Research Program

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QUESTIONS?

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Summary

Purpose is to show an association between jet fuel exposure and noise-induced hearing loss
JP-8 jet fuel believed to contributes to hearing loss when combined with high noise exposure that is still below the exposure limit for noise.

Objectives
- Design noise generation system for Navy Chambers
- Conduct 4 28-day animal studies
- Compare jet fuel exposures and kinetics

- Hearing loss represents a critical occupational concern for exposure to fuel
- Data suggest that Air Force personnel exposed to both jet fuel and noise suffer greater hearing loss than matched Air Force personnel exposed to similar noise but not to jet fuel

- State-of-the-Art noise generation system developed
- Used to determine noise level producing minimal hearing loss in rats
  - Combined noise and JP-8 study in progress
  - Delay in start of project has all milestones 6 to 12 months behind schedule

- Publication showing relationship between noise and jet fuel exposure expected in FY11
- Data would be relevant to determining need for applying protective measures for jet fuel/noise environments during occupational exposure for Air Force personnel

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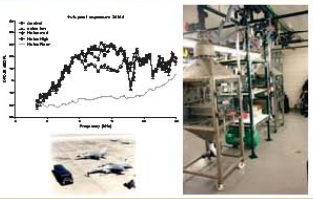
JP-8/Noise Hearing Loss
OAFWP001



Major Milestones

- Noise system developed by AFIT student for Navy exposure chambers
- Transitioned DPOAE hearing loss measurement system to Navy in collaboration with Dr. Fechter
- Completed noise-only exposure study
- Completed combined noise and jet fuel exposure study

Goals/Objectives

Goal
- To show if there is an association between jet fuel exposure and noise-induced hearing loss
- Hypothesis: JP-8 jet fuel contributes to hearing loss when combined with high noise exposure that is still below the exposure limit for noise.
- Need to provide well designed toxicology data

Objectives
- Design noise generation system for Navy chambers
- Conduct four 28-day animal studies
- Compare jet fuel exposures and kinetics

Applicability/AF Payoff

- Hearing loss represents critical occupational concern for exposure to fuel
- Data suggest that Air Force personnel exposed to both jet fuel and noise suffer greater hearing loss than matched Air Force personnel exposed to similar noise but not to jet fuel
- No clear results or definitive studies show an association between jet fuel exposure and noise
- Data would be relevant to determining need for applying protective measures for jet fuel/noise environments during occupational exposure for Air Force personnel

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Toxicity and Health Hazard Assessment for Synthetic Paraffinic Kerosene
711th Human Performance Wing (HPW)/RHPA

David Mattie, PhD

(Presented by Dr. John Hintz, U.S. Air Force School of Aerospace Medicine)

The U.S. Air Force is pursuing the development of alternative fuels. One jet fuel, designated as Synthetic Paraffinic Kerosene (SPK), is produced from natural gas using the Fischer-Tropsch (F-T) process. The toxicology experimental results for SPK showed that dermal irritation was slight to moderate and genotoxicity studies were negative. Results for the acute inhalation study, in which male and female rats were exposed to 2000 mg/m3 for 4 hours, revealed no abnormal clinical observations. In the two-week range finder study, male and female F344 rats were exposed for 6 hours per day, 5 days per week to an aerosol-vapor mixture of jet fuel (0, 500, 1000 or 2000 mg/m3). Based on results of the two-week study, male and female F344 rats were exposed for 6 hours per day, 5 days per week for 90-days to an aerosol-vapor mixture of 0, 200, 700 or 2000 mg/m3 SPK jet fuel. Histological findings in the nasal cavities were minimal (700 mg/m3) to mild (2000 mg/m3), while only the high dose (2000 mg/m3) produced multifocal inflammatory cell infiltration in rat lungs (both sexes). The 50% respiratory depression (RD50) value from the sensory irritation inhalation study was calculated to be 10,939 mg/m3. In a comparative health hazard assessment (HHA), these SPK results were compared to JP-8. SPK appeared moderately less toxic or irritating than JP-8 under similar exposure conditions. An Occupational Exposure Limit (OEL) for SPK was proposed to be 200 mg/m3, which is the current limit set for JP-8. Supported by AFMC 77 AESW/LF.

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Toxicology and Health Hazard Assessment for Synthetic Paraffinic Kerosene

Air Force Medical Research Symposium
25 August 2010

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Health Risk Assessment Toxicologist
USAFSAM/OEHRV
Air Force Research Laboratory

Outline

- CONTEXT: THE PAST AS PROLOGUE
  - Petroleum-based jet fuels – reviews, RA & Exposure Guidelines
  - Key Issues: The Challenge of Mixtures
- FISCHER-TROPSCH (SPK) PROGRAM
  - The Current Program
  - Toxicity Assessment
- SUMMARY: HRA & OEL
  - Respiratory Issues & Summary
  - OEL Recommendation
- FUTURE DIRECTIONS

JET FUEL: The Past as Prologue

- Industry & DOD
  - Wide variety of petroleum-derived fuels - 15 fuels identified
  - Important kerosene-based fuels: JP-8, JP-5, Jet A, Jet A-1
- Logistics, strategic security, energy independence
  - JP-8: DOD's "universal fuel": airplanes, tanks, stoves, heaters ...
  - HPV annual consumption:

	USAF	DOD	Civilian
B-Gals / yr	2.5	5	25

  - Augment with synthetic jet fuel(s) ... Fischer-Tropsch (SPK)
- Characteristics – petroleum-derived fuels
  - Properties/composition - vary by source & batch
  - F-T is synthesized - more controlled process
- The "Risk Assessment Challenge"
  - How to accommodate their variability & complexity?

CONTEXT: Prior Reviews

- WPAFB: THRU & AFRL technical research
  - Consortia w/ industry & academia
- ATSDR Toxicological Profiles (MRLs)
  - Two volumes: 1995 JP-4 & JP-7
    1998 JP-5 & JP-8
- Two USAFIOH-Sponsored Jet Fuel Conferences
  - Jet Fuel I (1999) – defined issues & research
  - Jet Fuel II (2001) – results → RA & RM
- Two NAS-COT PEL Assessments (mg/m^3)
  - 1996 USN JP-5/8/DFM: 350 & 1000 (STEL)
  - 2003 USAF JP-8: 200 (vapor) & 5 (aerosol)
- ACGIH TLV & AFOSH 48-8
  - Both guides referenced NAS-COT assessments
  - Incorporated aerosol/vapor issue & RD_{50}
- NAC- AEGL (1999-2009)
  - Guidelines - captured all previous work
  - AFIOH - generated Alarie/URT irritation data (RD_{50})



CONTEXT: The Challenge of Testing Jet Fuels

- Key 1st question: *What is the toxicity of the whole fuel?*
  - Questions addressing exposure scenarios are 2nd tier questions
- Issues
  - Complex mixtures with many components
  - Volatility varies across components → implications for saturation
  - Toxicity → component characteristic or a distributed property
  - Risk → fuel fractionation … older studies left constituents behind
- Procedures & techniques for *whole* fuel
  - Oral, dermal, etc.: *whole* fuel by direct application
  - Inhalation: atomize fuel → *mixed vapor / aerosol atmospheres*
    - dynamic atmospheres … vary with concentration
    - quantitate vapor & aerosol concentrations … saturation limits vapor conc.
    - incorporate into current toxicology & Alarie studies

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CONTEXT: Data Sets – Jet Fuels vs. F-T

	JP-5 & 8 Jet A & A-1	F-T	Data Gaps ?
Acute	+	✓	
URT Irritation	+	✓	
Sub-acute	-	✓	
Subchronic	+	✓	
Chronic	-	-	?
Cancer	-	-	?
Immunological	+	?	
Neurological	+	✓	
Developmental	+	-	?
Reproductive	+	"?"	?
Genotoxic	+	✓	

+ = data available; - = no data; ✓ = data now available

GOAL: comparative risk assessment, derivation of OEL

 6

Toxicity Assessment of Fischer-Tropsch (F-T) Jet Fuel (SPK)

 7

Toxicology of Synthetic Jet Fuel

- Background on SPK
- Development of the SPK toxicity program
- SPK toxicity program – Principle components
  - Dermal Irritation
  - Genotoxicity
  - Inhalation Studies

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BACKGROUND
Definitions & Origins & Goals

- **SPK (aka F-T, S-8…) = Synthetic Paraffinic Kerosene**
  - F-T = "Fischer-Tropsch"
  - S-8 = "Syntroleum-8"
- **USAF initiated the "alternative fuels" program in 1999**
  - Program sponsor
    - Alternative Fuels Certification Office (AFCO) [AFMC 77 AESW/LF]
    - MIL-HDBK-510 Aerospace Fuels Certification
      - Certify - fleet on alternative fuel by 2011
      - Fly - cost-competitively purchase 50% of domestic aviation fuel via "greener" alternative fuel blend by 2016
    - To be used as a "drop in" fuel for JP-8

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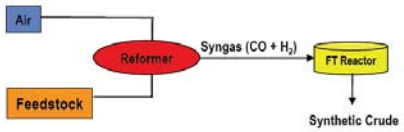
BACKGROUND
SPK – Synthesis

- Synthetic jet fuel derived by converting natural gas
  - Can also be made using coal gasification technology
  - Current synthetic jet fuels (S-8) are alkane-rich
    - Potential risk reduction – no aromatics/naphthalene



$(2n+1)H_2+nCO \rightarrow C_nH_{(2n+2)}+ nH_2O$

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BACKGROUND
Certification Status

- JP-8/SPK 50:50 Blend
  - ✓ B-52 Certified
  - ✓ C-17 Certified
  - ✓ B-1B Certified
  - ✓ F-15 Certified
  - ✓ F-22 , F/QF-4 and T-38 Certified after OEL proposed
  - ✓ C-5 and A-10 Certified in 2010
  - ✓ All fixed fuels infrastructure and GSE&V Certified
  - ✓ All USAF engines conforming to Mil-DTL-83133F
  - ✓ APUs certified
  - ✓ USAF Commercial-Derivative aircraft certification by FAA
  - 8 plane certifications in-process

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SPK
Program Development

- Funded by AFCO
- Goal - Complete initial toxicity tests by end FY08
- To ensure that toxicity program addresses all issues associated with testing a complex mixture, such as jet fuel, an expert review panel of toxicologists was established to discuss the toxicity testing program and provide advice on testing procedures.
- Health Hazard Assessment and recommended OEL by end FY09

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SPK
Program Development

- Expert Panel:
  - Chair:
    - **David Mattie, Ph.D., DABT, Chair, 711 HPW/RHPB**
  - Membership:
    - John Hinz, USAFSAM/OEHTH
    - Gunda Reddy, PhD, USACHPPM
    - LT Dean Wagner, PhD, MSC, USN, NHRC/EHEL
    - Katherine Kurtz, NMCPHC for HHA
    - David Steup, PhD, Shell Oil & Chairman, API-TTF*
    - Wayne Daughtrey, PhD, Exxon-Mobil for HHA*
    - Errol Zeiger, Ph.D., J.D., Errol Zeiger Consulting, Chapel Hill, NC

* AFRL has CRADA with API

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SPK
Program Development

- Develop appropriate study design for each toxicity test
- All testing → EPA / OECD test guideline & GLP compliant
- Test 100% SPK → maximize SPK's effects
- Study array:
  - ✓ Dermal irritation test (SPK *vs.* JP-8 *vs.* 50/50 blend)
  - ✓ *In vitro* genotoxicity tests
  - ✓ Acute inhalation study
  - ✓ Sub-acute inhalation rangefinder study
    - ✓ *In vivo* genotoxicity test in tandem with sub-acute
  - ✓ 90-day inhalation toxicity study
  - ✓ Sensory Irritation Assay (RD_{50})

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Characterizing SPK's Effects
Dermal Irritation/Toxicity

- Tested 100% SPK, JP-8 and 50/50 mixture SPK/JP-8
- Unoccluded and semi-occluded exposures (each animal its own control)
- Primary Irritation Index calculated to assign a descriptive rating to each test article and mixture

	JP-8	SPK	JP-8/SPK
Occluded			
Irritation Index	2.1	2.3	1.9
Descriptive rating	moderate	moderate	slight
Semi-occluded			
Irritation Index	1.8	0.8	1.5
Descriptive rating	Slight	slight	slight

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Characterizing SPK's Effects
In Vitro Genotoxicity

- **Ames Test (reverse mutation assay)**
  - Establish potential to induce point gene mutations in standard bacterial test in *S. typhimurium*: Does His(-) → His(+)?
  - Results
    - ✓ No toxic or mutation effects in any of five tester strains
    - ✓ No relevant increase in revertants
- **Chromosomal Aberration (CA) Test**
  - Establish potential to induce chromosomal aberrations in human lymphocytes
  - Results
    - ✓ No chromosomal aberrations induced
- **Conclusion**
  - ✓ **SPK is neither mutagenic nor clastogenic**

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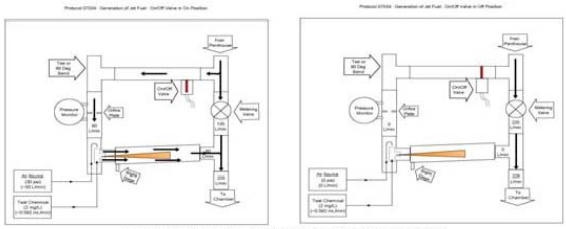
Characterizing SPK's Effects
Inhalation Toxicity

- The challenge of testing jet fuels – complex mixture with many components
  - Does toxicity relate to certain components or does it depend on the entire complex mixture?
- Program requirements for a complex mixture
  - Present all constituents → aerosolize fuel → aerosol/vapor mixture
  - Characterize vapor & aerosol partitioning, fingerprints, particle size



Characterizing SPK's Effects
Inhalation Toxicity

- Acute Inhalation Study
  - 4 hour exposure
  - 2000 mg/m^3 dose
  - 5 males / 5 females
  - No clinical symptoms observed

Characterizing SPK's Effects
Inhalation Toxicity

Two-week Study

- Rangefinder for 90-Day study + micronucleus assay

Group	Exposure Level	Number of Animals	
	mg/m^3	Males	Females
Control	0	5	5
Low	497.0 +/-8.4	5	5
Intermediate	999.0 +/-19.9	5	5
High	1958.1 +/-41.8	5	5
Micronuclei Neg Control	0	5	5
Micronuclei Pos Control	0	5	5
Total		30	30

Characterizing SPK's Effects
Two-week Inhalation Toxicity

Study Endpoints

- Observations before/after exposures for overt signs of toxicity
- Tissues and organs examined for gross pathology
  - nasal airways, trachea, larynx, lungs, liver, kidney, spleen, adrenals and heart
  - bone marrow (femur) for micronuclei analysis

Genotoxicity Results

- Micronuclei (MN) Induction Assay with Positive Control
  - Bone marrow examined
  - No micronuclei seen as a result of exposure
  - ✓Confirms SPK is non-genotoxic

Characterizing SPK's Effects
Two-week Inhalation Toxicity

General Observations/Results

- Body weight changes in high dose rats
- No change in food consumption
- All animals survived to necropsy

Necropsy Results

- Trachea, larynx, spleen, adrenals, heart → no adverse effects observed
- Liver - Hepatocyte hypertrophy → all exposed males
  → only highest dose females
- Kidney - Hyaline droplets (PCT) → all males exposed
  (male rat specific; not females/humans)

Characterizing SPK's Effects
Two-week Inhalation Toxicity

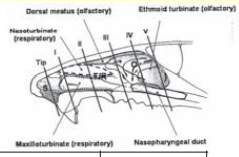
Necropsy Results
Lungs- Incidence of Inflammatory Cell Infiltration

Group [mg/m³]	Multifocal Inflammatory Cell Infiltration	Focal Inflammatory Cell Infiltration
Control [0] (Male)	0	0
Low [500] (Male)	0	0
Intermediate [1000] (Male)	1	1
High [2000] (Male)	**5**	**0**
Control [0] (Female)	0	0
Low [500] (Female)	0	0
Intermediate [1000] (Female)	0	2
High [2000] (Female)	**5**	**0**

Characterizing SPK's Effects
Two-week Inhalation Toxicity

Necropsy Results

- Nasal Cavity- Incidence (out of 5) of Olfactory Degeneration
- Severity: Minimal at 1000 mg/m³; Mild at 2000 mg/m³



Group [mg/m³]	Level II	Level III	Level IV
Control [0] (Male)	0	0	0
Low [500] (Male)	0	0	0
Intermediate [1000] (Male)	2	2	0
High [2000] (Male)	**4**	**5**	**5**
Control [0] (Female)	0	0	0
Low [500] (Female)	0	0	0
Intermediate [1000] (Female)	2	4	4
High [2000] (Female)	**5**	**5**	**4**

Characterizing SPK's Effects
90-Day Inhalation Toxicity

First of two primary studies for SPK's HHA & OEL

90-Day study design based on EPA & OECD test guidelines

- Full histopathology list plus
- Neurotoxicity Screening Battery (Motor Activity & FOB)
- Sperm morphology & vaginal cytology

Second of two primary studies for SPK's HHA & OEL

Sensory Irritation Assay

- ASTM E-981-04 – standardized procedure, derived from Alarie (1973)
- Based on dose / response for breathing rate depression
- Alarie established correlation with established TLVs → OEL

Characterizing SPK's Effects
90-Day Inhalation Toxicity

Group *	Exposure Level	Number of Animals	
	mg/m³	Males	Females
Control	0	5	5
Control		5	5
Low Dose	200	5	5
Low Dose		5	5
Intermediate Dose	700	5	5
Intermediate Dose		5	5
High Dose	2000	5	5
High Dose		5	5
Total		40	40

* Staggered groups to allow sufficient time to collect data at end of exposure.

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Characterizing SPK's Effects
90-Day Inhalation Toxicity

Observations & Results

- Animals observed before and after exposures for overt signs of toxicity - BW decrease only at 2000 mg/m³
- Tissues and organs examined for gross pathology – no biologically significant changes
- Blood collected for clinical pathology – no biologically significant changes
- Full tissue list collected for histopathology
- Analyzed male rat kidneys for alpha 2 microglobulin
  - present, but SPK weak inducer compared to JP-8

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Characterizing SPK's Effects
90-Day Inhalation Toxicity

Observations/Results

- Full tissue list collected for histopathology – only significant changes in **nasal cavities** and lungs in 2000 mg/m³ rats

Group (**mg/m³**)	Olfactory Epithelial Degeneration		Hypertrophy/Hyperplasia of Goblet Cells, Nasopharyngeal Duct	
	Incidence/Severity		Incidence/Severity	
	Male	Female	Male	Female
Control (0)	0	0	0	0
Low (200)	0	0	0	0
Intermediate (700)	9/minimal	8/minimal	10/minimal	9/minimal
High (2000)	**10/slight-mild**	**10/slight-mild**	**10/slight-mild**	**10/slight-mild**

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Characterizing SPK's Effects
90-Day Inhalation Toxicity

Observations/Results

- Full tissue list collected for histopathology – only significant changes in nasal cavities and **lungs** in 2000 mg/m³ rats

Group (**mg/m³**)	Multifocal Inflammatory Cell Infiltration		Focal Inflammatory Cell Infiltration	
	Incidence		Incidence	
	Male	Female	Male	Female
Control (0)	0	0	0	0
Low (200)	0	0	1	0
Intermediate (700)	0	0	1	3
High (2000)	**10**	**10**	**0**	**0**

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Characterizing SPK's Effects
90-Day Inhalation Toxicity

Observations & Results (cont.)

- **Motor Activity and Functional Observational Battery**
  - Assessment of neurobehavioral effects
    - Only changes in 2000 mg/m^3 rats
- **Sperm Morphology and Vaginal Cytology Examinations**
  - Sperm stained and examined for percentage of abnormal sperm
    - No effects seen
  - Potential alterations in the estrous cycle assessed by examination of cells from vaginal smears
    - No effects seen

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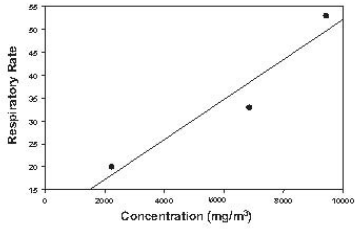
Characterizing SPK's Effects
URT Sensory Irritation

Administration: vapor/aerosol mixture, 30 min (+) recovery

- Establish dose/response for RD_{50}
- Multiple groups of male mice - 4 mice per group
- Exposed for 30 minutes
- Compare with JP-8 data



RD_{50} (mg/m^3):

SPK	vs.	JP-8
10,939		**2,876**

Higher value means less irritating

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Characterizing SPK's Effects
Conclusions

- **Dermal: slightly to moderately irritating – same as JP-8**
- **Not Genotoxic: two *in vitro* and one *in vivo* studies**
- **Inhalation**
  - Acute - No clinical symptoms observed at 2000 mg/m^3
  - 2-week - Histological findings in:
    - ✓ Lung: inflammatory foci evident in highest doses
    - ✓ Olfactory epithelia: degeneration in highest doses
    - ✓ Kidney: hyaline droplet accumulation in all male rats exposed (not relevant to human kidneys)
    - ✓ Liver: hepatocyte changes not seen after 90-days
  - 90-day
    - ✓ Significant changes limited to highest dose
    - ✓ 200 mg/m^3 was a no effect level
  - Sensory Irritation
    - ✓ Less of an irritant than JP-8

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HRA & OEL

Respiratory Issues & Summary

Recommendation

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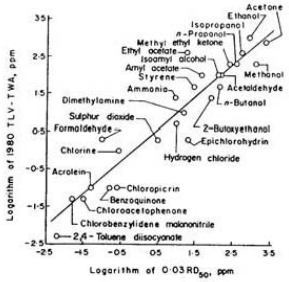
Upper Respiratory Tract Sensory Irritation

ASTM: Standard Method 1981-84

- **Derived from Prof. Yves Alarie's Assay (1966-82)**
  - Method to detect effects *before* damage is done
  - Reflex-induced modification of respiration
  - Simple, reproducible, quantitative measure of irritancy
    - Based on exposure concentration vs. breathing rate
    - Describe dose-response & median effective dose → RD_{50}
- **Application**
  - Identify respiratory & eye irritants
  - Comparative ranking
  - Propose OELs … must be in context w/ health effects/ toxicology!
  - Many industrial chemicals tested … including jet fuels

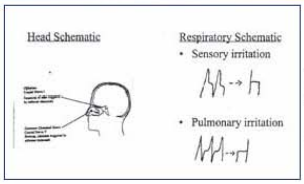
ALARIE (1981): Correlation of TLV vs. (3%)RD_{50}

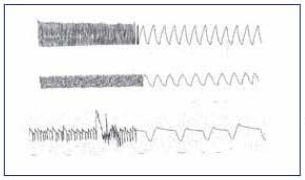


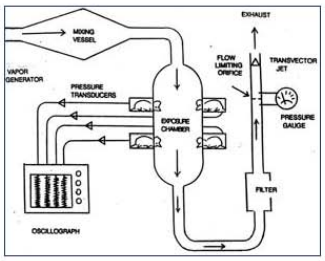
Observation: Prevailing TLVs already set at 1% - 10% of RD_{50}

Baseball analogy: 1% & 10% = left & right field foul lines
3% = hit onto the field of play
Apply rest of toxicology to define score

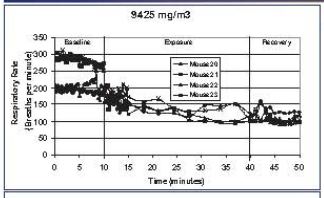
SCHEMATICS - sensory vs. pulmonary irritation





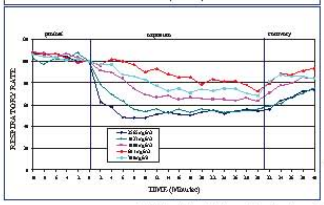


RD_{50} Data Summary



F-T

RD_{50}: JP-4, JP-8, JP-8+100, JP-5



"Limit Test": JP-7, JPTS, JP-10, DFM

JP-8

CONTEXT: ASTM Method E981-84 → Applied To Various Jet Fuels

RD_{50} Data Sets

FUEL	JP-4	JP-5	JP-8+100	JP-8	F-T
RD_{50}	4842	3338	1629	2876	10,939
lfffl - - - -1%	48	33	16	29	109
hit → 3%	145	100	49	86	328
rfffl - - -10%	480	334	163	288	1094
max vapor	-	804	-	708	736
PEL (mg/m^3)				200	*"200"*
aerosol				5	*"5"*
STEL / IDLH	1000 / 1800				*"1000"*

CONTEXT: NAC-AEGL & ACGIH TLV & AFOSH 48-8

- **NAC-AEGL**
  - Three degrees of severity across five time periods (mg/m^3)

AEGL-I: 290	AEGL-II: 1100	AEGL-III: ND
RD_{50}	"WOE" – 6 studies	no mortality – no data

  - Public comment → peer review → finalized by NAS-COT
- **ACGIH TLV for kerosene & jet fuel**
  - Broad survey - captures COT (1996), AFIOH RD_{50}
  - Guideline: 200 mg/m^3 as vapor; no STEL or IDLH
  - Warnings: skin and respiratory irritation, aerosols more irritating
- **AFOSH 48-8**
  - Incorporates NAS-COT, AFIOH, ACGIH evaluations

OEL TWA (mg/m^3)	STEL (mg/m^3)
200	1800

COMPARATIVE ASSESSMENT: JP-8 vs. F-T

AEGL (mg/m^3)	Level-I	Level-II	Level-III
JP-8	290	1100	NR (no mortality)

	JP-8	F-T
RD_{50} (mg/m^3)	2876	10939
lffl → 1%	29	109
3%	86	328
rffl → 10% (threshold)	288	1094
max vapor	708	736

		(mg/m^3)
F-T	NOAEL (2 wk - subchronic)	200 - 500
	LOAEL (2 wk - subchronic)	700 - 1000
	Uncertainty range 3-10	20 - 333
JP-8	NAS-COT Guidance	5 / 200 (aerosol / vapor)

PROGRAM SUMMARY

- **F-T / SPK Program**
  - Assembled multi-disciplinary team → defined key data
  - Toxicology studies: GLP & test guideline compliant
  - Developed F-T/SPK data base comparable to JP-8
- **Findings**
  - Dermal Irritation: SPK =/< JP-8
  - Genotoxicity: Not genotoxic
    - neither mutagenic nor clastogenic
    - not likely to be frankly carcinogenic
  - Inhalation Toxicity
    - acute – unremarkable
    - 2-wk study – effects minimal-mild, NOAEL 500-1000 mg/m^3
    - subchronic – effects minimal-mild, NOAEL 200-700 mg/m^3 (sperm morphology/vaginal cytology unremarkable)
    - Potential for respiratory irritation – less than JP-8

RECOMMENDATION: F-T & 50/50 Blend w/ JP-8

- Flight Certification
  - 50/50 blend of F-T / JP-8
- Based on comparative toxicity, F-T vs. JP-8
  - F-T is comparable to / less toxic or hazardous than JP-8
- For safety's sake – conservative approach
  - Let the "more hazardous" fuel define OEL & ESOH guidance
- Adopt JP-8's OEL and ESOH practices – established, familiar
- Apply to both blended fuels & pure F-T – prudent, protective
- Preparation of OEL guidance / ESOH policy begins at USAFSAM

FUTURE DIRECTIONS: Preparing Guidance & Policy

- AFOSH 48-8
  - "Umbrella" for specific OELs
  - Rescinded … guidance still needed
- USAFSAM Mission → guidance governing use/exposure
  - Draft HRA & OEL guidance
    - Kerosene-based fuels
    - F-T / SPK derived fuels
    - Other synthetic fuels
  - Capture requirements for informed review → guidance
  - Draft guidance → predicate for ESOH policy
- Policy requires SG concurrence

FUTURE DIRECTIONS: Bio Derived Fuels - Issues

- Biomass – many sources/products for deriving bio derived fuels
- Time frame – from development to certification - critical
  - EPA: 5-10 years; USAF needs results in 2-3 years
- The challenge of testing & certification
  - Too many fuels to conduct FT program for *every* fuel
  - Need methods & process to conduct analytical screen of new fuels
    - Comparison of chemical components base on GC/MS
  - Identify the appropriate toxicity tests as screen for new fuels
    - NAS recommendations (1996 & 2003)
    - REACH Program – Europe
    - Toxcast Program – USEPA
    - Existing health hazard assessments for JP-8 & F-T as context
  - Identify relevant endpoints as predictors for new fuel HHA & OEL
- New bio derived fuels – "Hydrotreated Renewable Jet" (HRJ)
  - Two new fuels to be certified by AFCO
  - Both fuels need HHA & OELs

QUESTIONS?

Cellular Bioeffects Thresholds for Terahertz Frequency

711th Human Performance Wing (HPW)/RHDR/AFRL 8262 Hawks Rd, Brooks City-Base, TX 78235

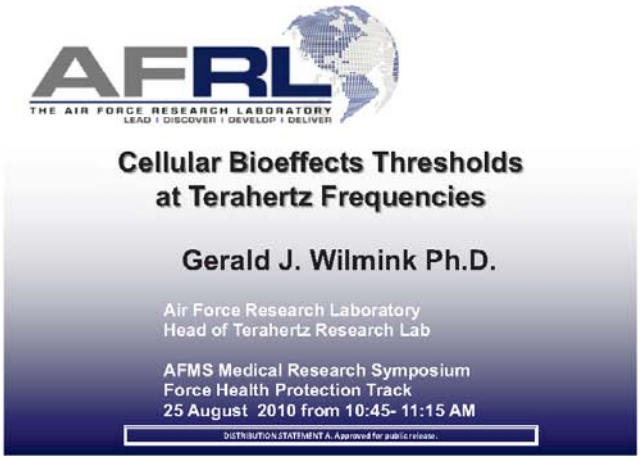
Gerald Wilmink, DRII/Biomedical Engineer

The Terahertz (THz) region of the electromagnetic (EM) spectrum is defined as frequencies ranging from 0.1 to 10 THz. Historically, few sources have been available for this region; however, in recent years, several advances have been made in THz source development. Such advances have enabled numerous "real world" applications. For instance, THz techniques are now being used for security purposes to identify concealed explosives, drugs, and weapons. However, despite efforts to develop these applications, the bio-effects associated with THz radiation are not well characterized.

In this study, we used computational and empirical approaches to investigate the biological effects associated with THz radiation at a tissue, cellular, and molecular level. To examine THz-tissue interactions we conducted the following: (1) Developed THz- spectroscopy system to measure the optical properties of biological tissues; (2) Determined tissue-damage thresholds (ED50) using a Free Electron Laser and a molecular pumped THz source; and (3) Developed computational modeling algorithms to predict dosimetry and damage thresholds.

To examine THz-cellular effects we conducted the following: (1) Developed computational models to predict dosimetry and cell death-thresholds for THz-exposed cells; (2) Empirically determined cell-death thresholds using a THz laser, infrared camera, MTT assays, flow cytometer, confocal laser scanning microscope, and several adherent and suspension cell lines (e.g. Hela, NHDF, Jurkat).

Last, to examine THz-biomolecular effects, we used molecular dynamics modeling and empirical approaches. Specifically, we used genomic and transcriptomic analysis techniques (microRNA/mRNA microarray gene chips and qPCR) to characterize the cell's molecular response to THz radiation.


AFRL
THE AIR FORCE RESEARCH LABORATORY
LEAD | DISCOVER | DEVELOP | DELIVER
Cellular Bioeffects Thresholds at Terahertz Frequencies
Gerald J. Wilmink Ph.D.
Air Force Research Laboratory
Head of Terahertz Research Lab
AFMS Medical Research Symposium
Force Health Protection Track
25 August 2010 from 10:45- 11:15 AM
DISTRIBUTION STATEMENT A. Approved for public release.


Meet the Speaker
My flight to the Air Force
Biomedical Engineering Optics
Biomedical Engineering
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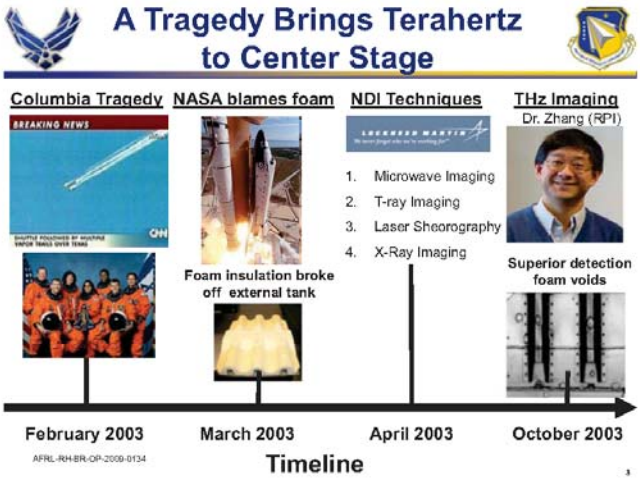
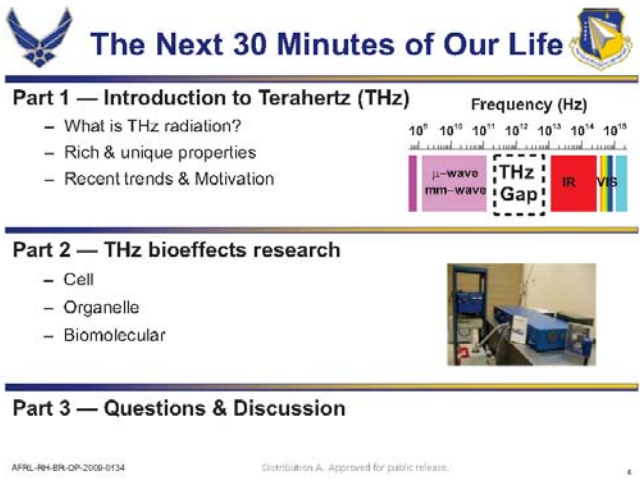

A Tragedy Brings Terahertz to Center Stage
Columbia Tragedy
NASA blames foam
NDI Techniques
THz Imaging
Dr. Zhang (RPI)
1. Microwave Imaging
2. T-ray Imaging
3. Laser Sheorography
4. X-Ray Imaging
Foam insulation broke off external tank
Superior detection foam voids
February 2003
March 2003
April 2003
October 2003
Timeline
AFRL-RH-BR-OP-2009-0134


The Next 30 Minutes of Our Life
Part 1 — Introduction to Terahertz (THz)
– What is THz radiation?
– Rich & unique properties
– Recent trends & Motivation
Frequency (Hz)
μ–wave
mm–wave
THz Gap
IR
VIS
Part 2 — THz bioeffects research
– Cell
– Organelle
– Biomolecular
Part 3 — Questions & Discussion
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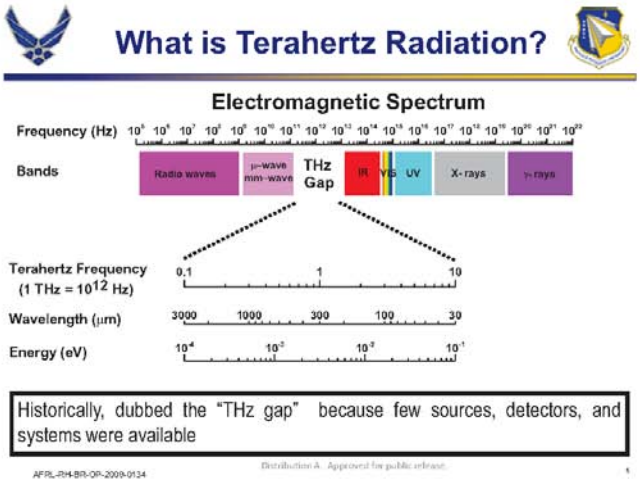
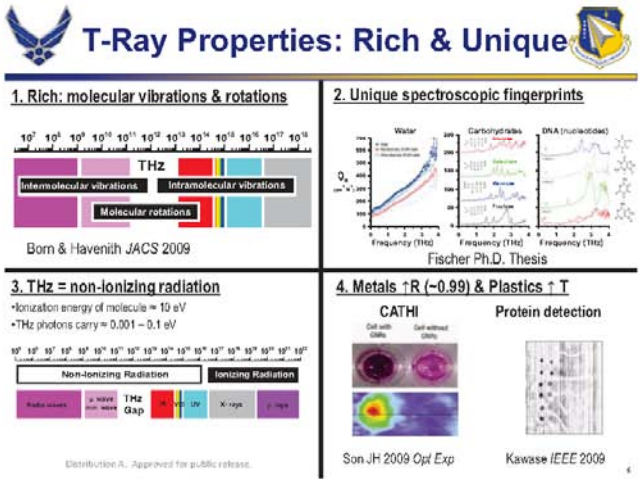
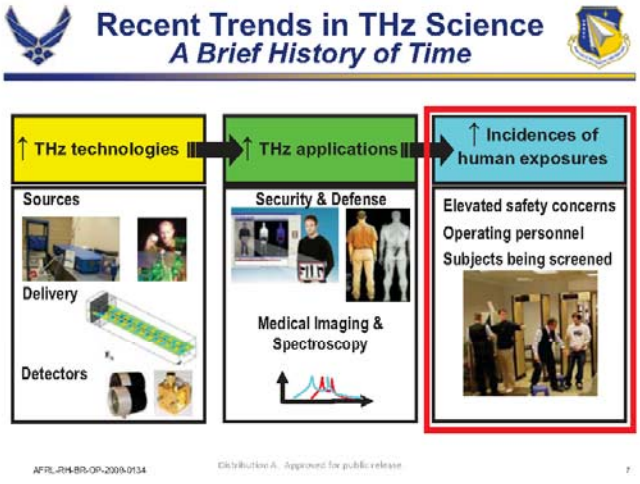
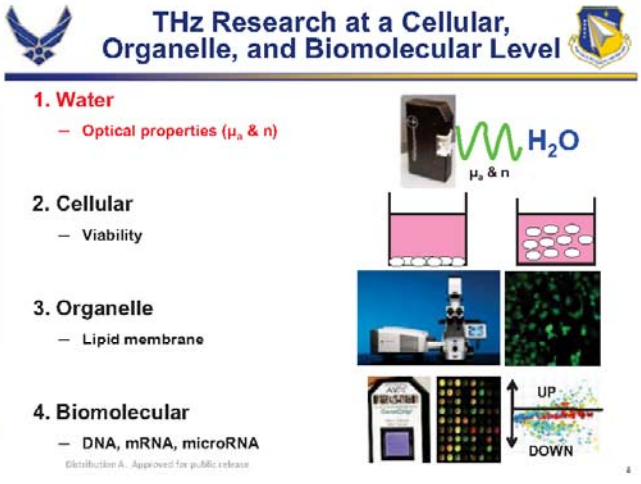
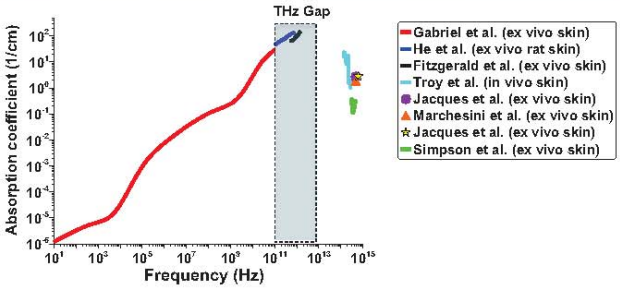

What is Terahertz Radiation?
Electromagnetic Spectrum
Frequency (Hz)
Bands
Radio waves
μ-wave
mm-wave
THz Gap
IR
VIS
UV
X-rays
γ-rays
Terahertz Frequency
(1 THz = 10^12 Hz)
0.1
1
10
Wavelength (μm)
3000
1000
300
100
30
Energy (eV)
Historically, dubbed the "THz gap" because few sources, detectors, and systems were available
AFRL-RH-BR-OP-2009-0134


T-Ray Properties: Rich & Unique
1. Rich: molecular vibrations & rotations
THz
Intermolecular vibrations
Intramolecular vibrations
Molecular rotations
Born & Havenith JACS 2009
2. Unique spectroscopic fingerprints
Water
Carbohydrates
DNA (nucleotides)
Frequency (THz)
Fischer Ph.D. Thesis
3. THz = non-ionizing radiation
•Ionization energy of molecule ≈ 10 eV
•THz photons carry ≈ 0.001 – 0.1 eV
Non-Ionizing Radiation
Ionizing Radiation
THz Gap
4. Metals ↑R (~0.99) & Plastics ↑ T
CATHI
Protein detection
Son JH 2009 Opt Exp
Kawase IEEE 2009


Recent Trends in THz Science
A Brief History of Time
↑ THz technologies
↑ THz applications
↑ Incidences of human exposures
Sources
Delivery
Detectors
Security & Defense
Medical Imaging & Spectroscopy
Elevated safety concerns
Operating personnel
Subjects being screened
AFRL-RH-BR-OP-2009-0134


THz Research at a Cellular, Organelle, and Biomolecular Level
1. Water
– Optical properties (μa & n)
H2O
μa & n
2. Cellular
– Viability
3. Organelle
– Lipid membrane
4. Biomolecular
– DNA, mRNA, microRNA
UP
DOWN

Project #1: Motivation & Goal



Goal for Study: Develop a Terahertz Time-Domain Spectrometer to Measure the Optical Properties of Water Using

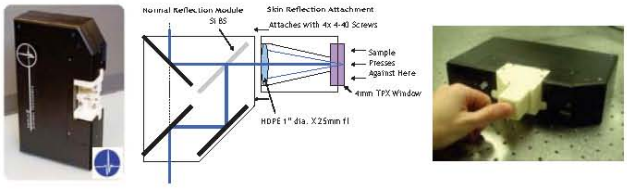
 9

A Collaborative Research Approach

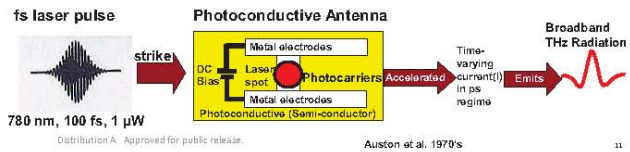
Dr. Wilmink Dr. Ibey Mr. Tongue Dr. Schulkin Dr. Zhang Dr. Peralta

 10

A Compact THz-Time Domain Spectrometer for Skin Measurements



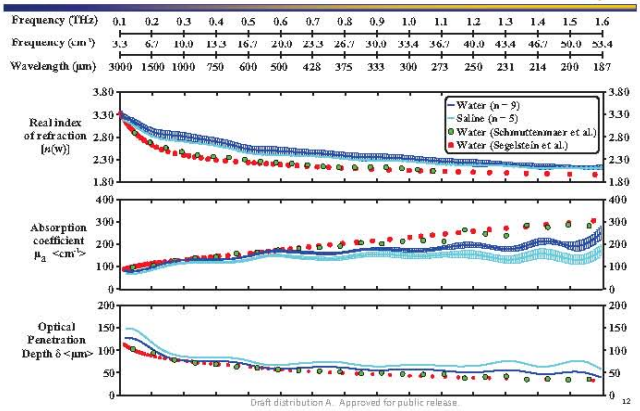
How the source works? Transient Photoconductive Switching



Auston et al. 1970's

 11

Optical Properties of Water at THz Frequencies



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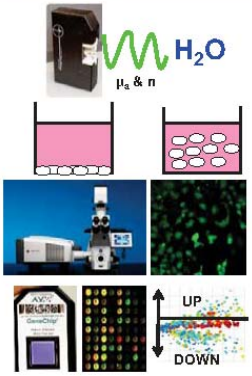
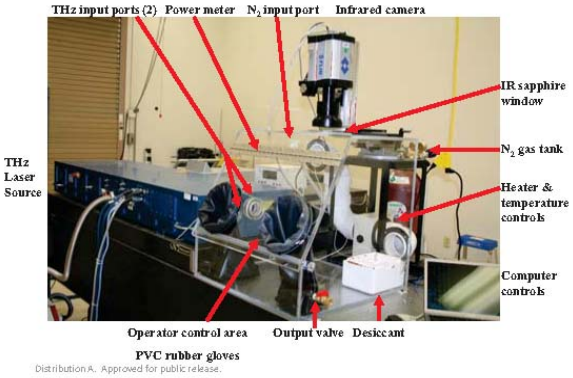
THz Research at a Cellular, Organelle, and Biomolecular Level

1. Water
   - Optical properties (μ_a & n)
2. Cellular
   - Viability
3. Organelle
   - Lipid membrane
4. Biomolecular
   - DNA, mRNA, microRNA



Distribution A. Approved for public release.

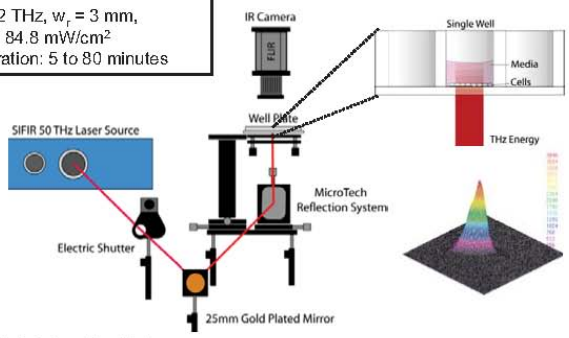
in vitro THz Exposure Setup



Distribution A. Approved for public release.

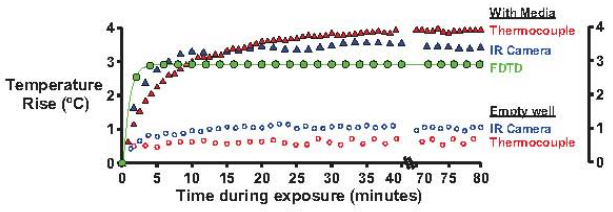
in vitro THz Irradiation of Dermal Fibroblasts

THz Source Parameters
2.52 THz, w_r = 3 mm,
H = 84.8 mW/cm²
Duration: 5 to 80 minutes



Distribution A. Approved for public release.

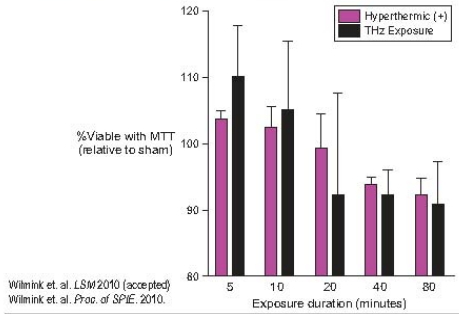
Dosimetry Data



Wilmink et. al. *LSM* 2010 (accepted)
Wilmink et. al. *Proc. of SPIE.* 2010.

Distribution A. Approved for public release.

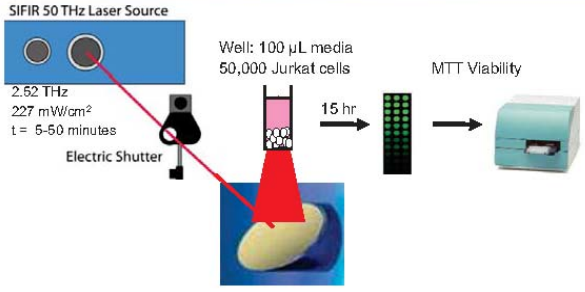
Viability of NHDFs Exposed to THz Radiation



Wilmink et. al. *LSM* 2010 (accepted)
Wilmink et. al. *Proc. of SPIE*. 2010.

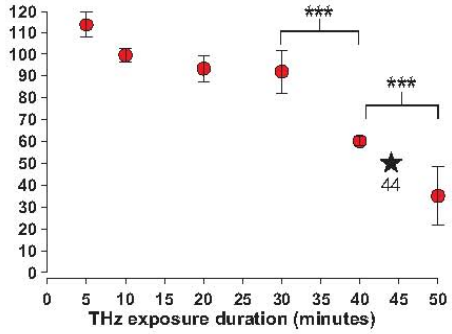
Take Home Message: THz & hyperthermic-exposed cells exhibit comparable levels of viability—appears damage governed by photothermal mechanisms

in vitro THz Irradiation of Jurkat Cells (suspension)



Take Home Message: Improvements to our experimental techniques (focusing, purging, cycloolefine plates are superior to polystyrene plates (↑T & ↓n)

Viability Data for Jurkat Cells

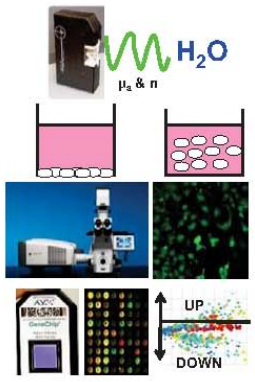


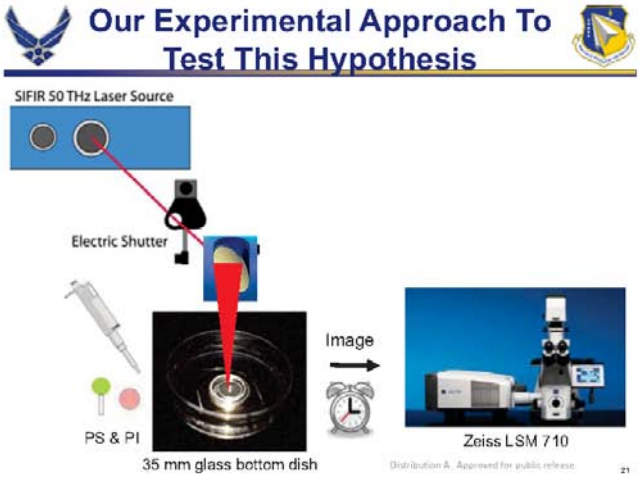
Take Home Message: ED_{50} for Jurkat Cells is 44 minutes (2.52 THz, 227 mW/cm²)

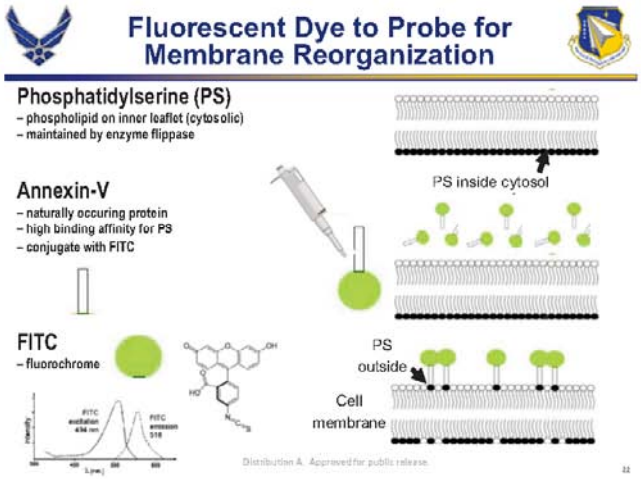
Grundt J. et. al. BEMS (in preparation)

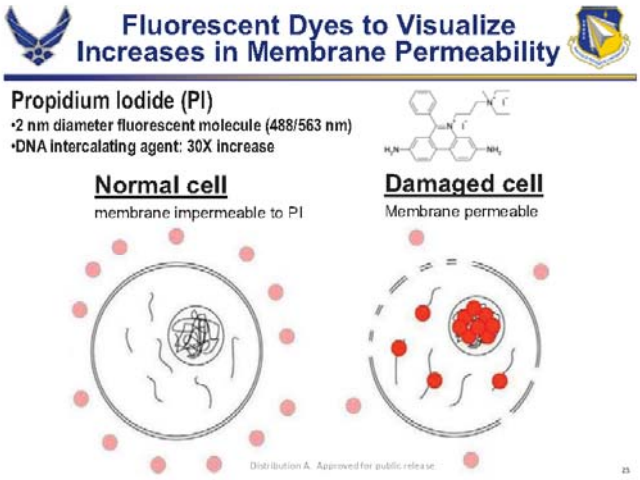
THz Research at a Cellular, Organelle, and Biomolecular Level

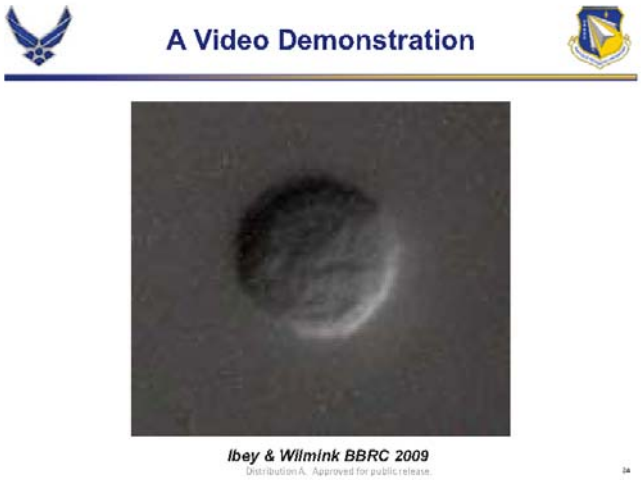
1. **Water**
   - Optical properties (μ_a & n)
2. **Cellular**
   - Viability
3. **Organelle**
   - Lipid membrane
4. **Biomolecular**
   - DNA, mRNA, microRNA



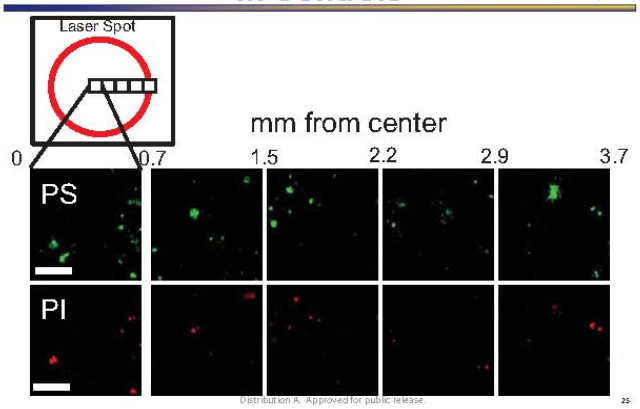

Our Experimental Approach To Test This Hypothesis
SIFIR 50 THz Laser Source
Electric Shutter
PS & PI
35 mm glass bottom dish
Image
Zeiss LSM 710


Fluorescent Dye to Probe for Membrane Reorganization
Phosphatidylserine (PS)
– phospholipid on inner leaflet (cytosolic)
– maintained by enzyme flippase
Annexin-V
– naturally occuring protein
– high binding affinity for PS
– conjugate with FITC
FITC
– fluorochrome
PS inside cytosol
PS outside
Cell membrane

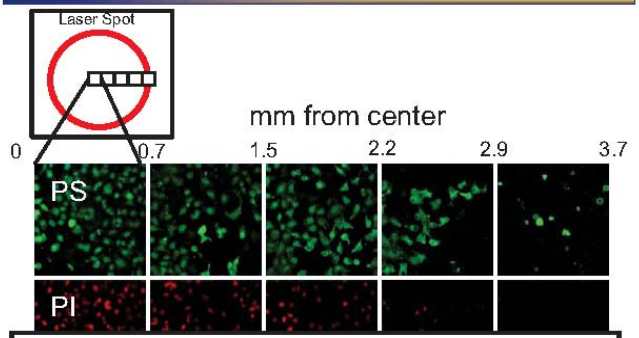

Fluorescent Dyes to Visualize Increases in Membrane Permeability
Propidium Iodide (PI)
•2 nm diameter fluorescent molecule (488/563 nm)
•DNA intercalating agent: 30X increase
Normal cell
membrane impermeable to PI
Damaged cell
Membrane permeable


A Video Demonstration
Ibey & Wilmink BBRC 2009

Membrane Is Unaffected In Controls



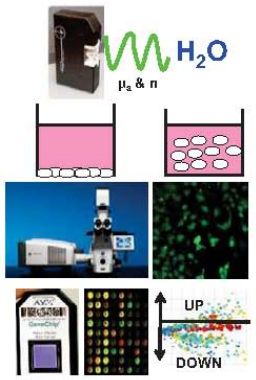
Fluorescence Images THz-exposed fibroblasts



Take Home Point: THz exposed cells exhibit both membrane reorganization and increased permeability

THz Research at a Cellular, Organelle, and Biomolecular Level

1. **Water**
   - Optical properties (μ_a & n)
2. **Cellular**
   - Viability
3. **Organelle**
   - Lipid membrane
4. **Biomolecular**
   - DNA, mRNA, microRNA



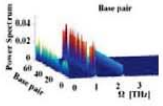
A Subject of Constant Debate—

There are people who believe in nonthermal effects and there are people who don't.....

Non-thermal effects

Driven collection of vibrational oscillators can condense with all energy activating mode of lowest frequency (Bose-Einstein Condensation)
- **Frohlich *JQC* & *Phys L.A.* 1968**

THz radiation can couple directly to the vibrational modes of biomolecules via coherent excitations (linear/nonlinear resonances), thereby causing nonthermal or microthermal effects
- **Frohlich *Phys L.* 1975**
- **Korenstein-Ilan *Rad Res* 2008**



- **Alexandrov *Bio Phys.* 2009**
- **Reimers *PNAS.***

Thermal effects

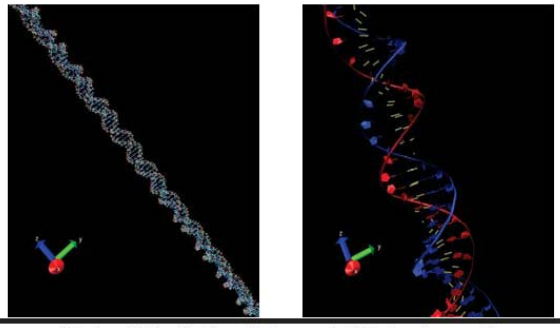
Give us unambiguous experimental proof of "Frohlich Condensation"

To date, no experimental evidence

Can low-power EM radiation disrupt hydrogen bonds in dsDNA?
No— Chitanvis. *J Poly Science.* 2006

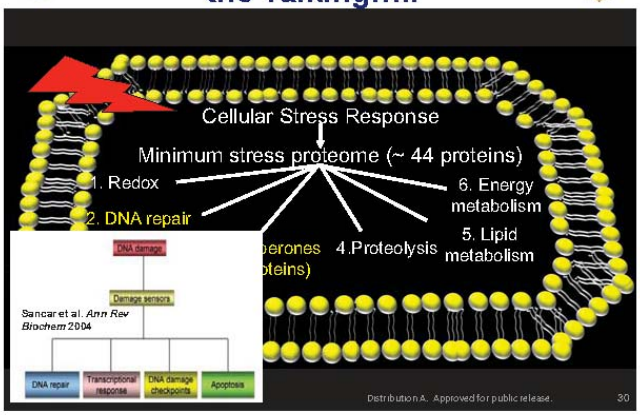
THz exposure is not able to induce either genotoxicity in human blood cells
No— Zeni. *Health Physics.* 2007

Modeling with NAMD (NAnoscale Molecular Dynamics) on Super Computers



However, this is at 0K, effects not observed at higher temperatures

Our Approach—Let The Cell Do the Talking…..

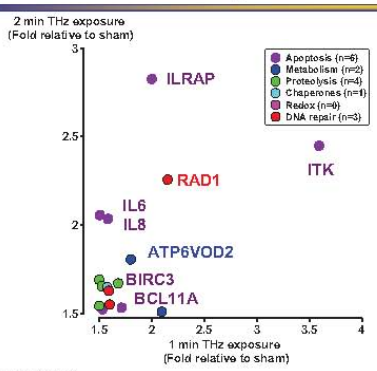


The Details of Our Empirical Approach

1. **Expose NHDFs to 2.52 THz—frequency cited for targeting natural breathing mode of DNA**
   - **H = 227 mW/cm^2—roughly 7,500X higher prior studies**
2. **Examine genetic profile of cells**
   - **Most sensitive technique to quantify alterations to DNA**

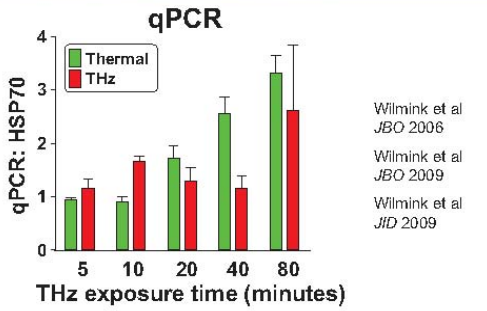
Affymetrix Gene Chip
- HG-U133 Plus 2.0
- 54,576 genes

Gene Expression for Known Pathways



Wilmink, G.J. et al. *Proc. SPIE.* 2010.
Wilmink, G.J. et al. BEMS (in preparation)

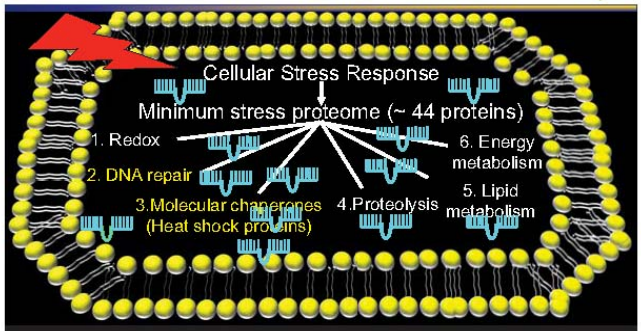
Comparing THz and Hyperthermic Exposed NHDFs



Take Home Message: THz & hyperthermic-exposed cells exhibit comparable levels of protein specific damage mechanisms, which suggests THz is not causing additional protein damage

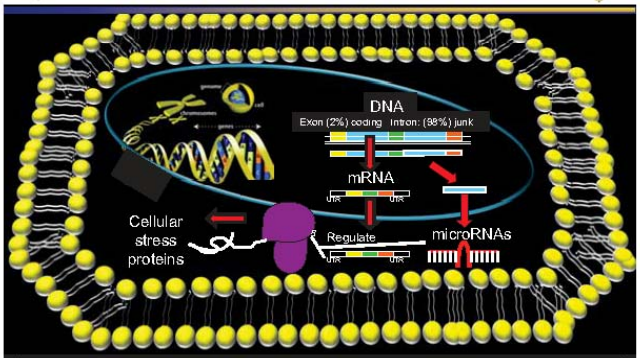
Minimal Stress Response

at least what was known about it…



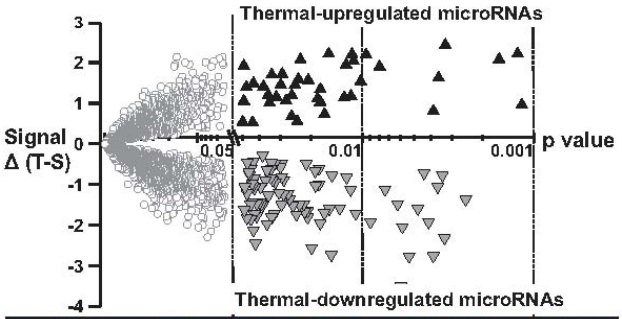
Pathways and minimal stress proteins involved in CSR well known; however, small RNAs were upregulated!!!!!

Hidden Layer of Internal Signals Controlling Gene Expression



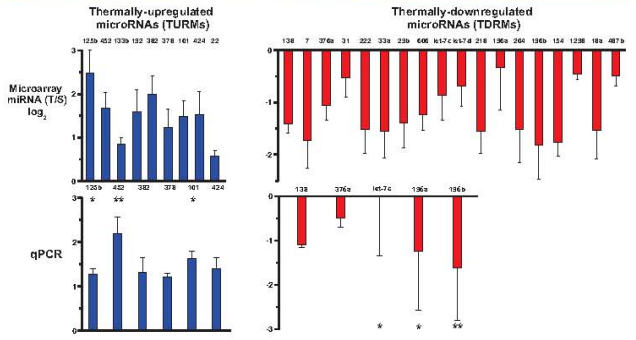
MicroRNAs are upstream from common cellular stress mRNA markers, and actually regulate their expression

Identified a New Family of Thermally Regulated miRNAs (coined the name "TRMs")



	miRNA	P > .05	.05 ≥ P ≥ .01	.01 ≥ P ≥ .001	Totals
+	1417	1377	32	8	40
-	1870	1787	69	14	83
Sum	3287	3164	101	22	123

TRMs: qPCR Validation



Wilmink GJ *et.al. Cell Stress and Chaperones.* 2010
Wilmink GJ *et.al. Proc. of SPIE.* 2009

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Research Summary

Water & Cell
- THz-TDS device measure optical properties water & tissues
- Future work- *in vivo* human studies (IRB approval)
- Lethal thresholds several cell lines

Organelle
- Membrane reorganization
- Increases in permeability

Biomolecular
- Microarray data: DNA repair and inflammatory genes expressed
- Identified TRMs, examine miRNA response in THz-exposed cells

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Acknowledgements

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Funding Agencies

NSF
UTSA
AFOSR
AIR FORCE OFFICE OF SCIENTIFIC RESEARCH

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Development of a Health-Belief-Model-Based Instrument to Assess Worker Beliefs about using PPE

Utah Air National Guard

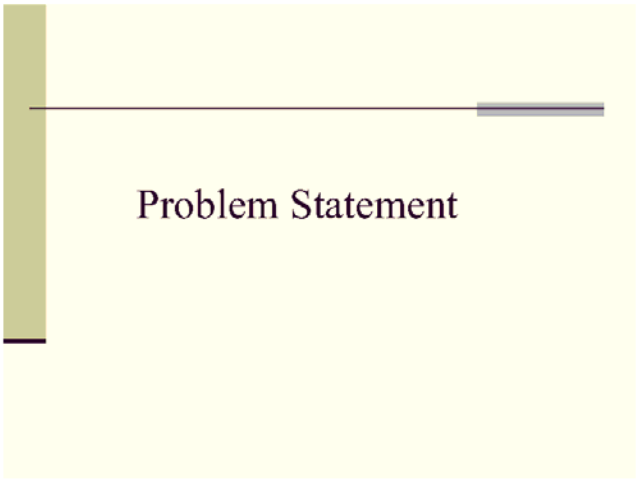
LTC Jack Wall

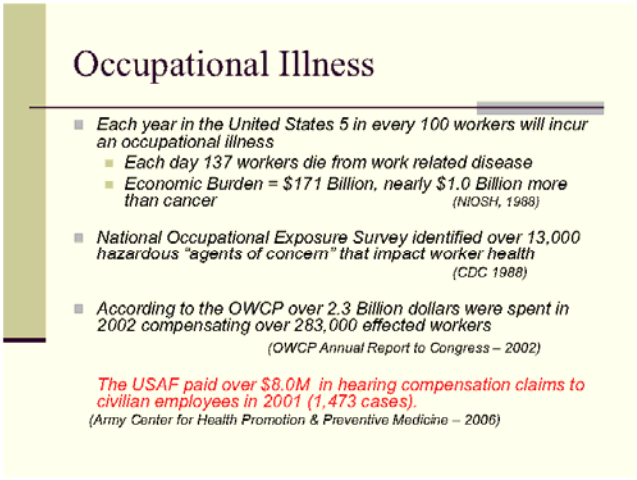
Occupational illness is an identified problem in the United States Air Force (USAF). Of the many occupational illnesses reported annually, most are preventable through the use of personal protective equipment (PPE). The purpose of this study was to develop an instrument to assess the significance of the determinants that predict the use of PPE in small industrial USAF shops. The focused aim of the study was to develop a valid and reliable theory-driven instrument, specific to the military, assessing these determinants resulting in effective interventions. The health belief model was used as the theoretical basis for the instrument.

The procedures employed USAF expert and employee focus groups to establish instrument validity. A two-judge content validity index was calculated using judges from the expert focus group. Reliability was established by test-retest administration of the instrument. An analysis of Cronbach's alpha was used to assess the test-retest reliability of the health belief model constructs.

The focus groups established that the instrument is valid. Reliability of the instrument varied by construct, with the majority of the constructs having sufficient reliability to make the instrument useful for assessing determinants of behavior contributing to the use of PPE. More research is recommended to enhance the reliability of the instrument and to demonstrate equal value in the non-military situation. The developed instrument fills a need for theory-based instruments that can be used to plan theory-driven interventions that target increasing appropriate PPE use.


AFMS Research Symposium
Developing an Instrument to Assess Worker Beliefs About Using Personal Protective Equipment (PPE)
LTC Jack M. Wall, 151 MSG/CC
25 August 2010


Problem Statement


Occupational Illness
Each year in the United States 5 in every 100 workers will incur an occupational illness
Each day 137 workers die from work related disease
Economic Burden = $171 Billion, nearly $1.0 Billion more than cancer
(NIOSH, 1988)
National Occupational Exposure Survey identified over 13,000 hazardous "agents of concern" that impact worker health
(CDC 1988)
According to the OWCP over 2.3 Billion dollars were spent in 2002 compensating over 283,000 effected workers
(OWCP Annual Report to Congress – 2002)
The USAF paid over $8.0M in hearing compensation claims to civilian employees in 2001 (1,473 cases).
(Army Center for Health Promotion & Preventive Medicine – 2006)


Small Shop Focus
Defined as those with 10 or fewer employees
Comprises over 98% of private industry
Employs over 50% of U.S. Private industry workforce (approx 53 million workers)
Typically lack resources to properly protect workers
Not well regulated
(Okun et al. 2001)

Body Area Protection Focus

(Excludes Repeated Trauma Disorders)

- Skin Protection
  - Contact Dermatitis – Cancer
  - 36% of all occupational illness
- Respiratory Protection
  - Lungs & Airway
  - Respiratory conditions such as asthma and Cancer
  - 16% of all occupational illness
- Hearing Protection
  - Tinnitus (ringing in ears)
  - Hearing Loss (OSHA Special Category)
  - 11% of all occupational illness

(BLS 1997 and McCall et al. 2005)

PPE Definition

- **Personal Protective Equipment (PPE)** includes all clothing and other work accessories designed to create a barrier against workplace hazards.
  - safety goggles,
  - hard hats,
  - hearing protectors,
  - gloves,
  - respirators,
  - aprons,
  - work boots.

(OSHA 29 CFR 1910)

Preventable Occupational Diseases and Required PPE

Preventable Disease	Personal Protective Equipment
Skin Disorders Dermatitis Cancers	Barriers - i.e. gloves, aprons
Respiratory Illnesses Interstitial Fibrosis Asthma Bronchitis Cancer	Respiratory Protection Respirators Dust Masks
Hearing and Neurological Disorders Hearing Loss Encephalopathy	Hearing Protection Ear Plugs Ear Muffs
Infectious Diseases Blood borne (HIV, Hep B) TB Hep A	Universal Precautions Mask Gloves Over clothes

(Lax, Minetti, Klein, 1998)

Should I Get One?

Literature Review

Importance of PPE

- Permissible Exposure Limits (PEL) for each chemical set by NIOSH (NIOSH 1996)
- PPE use mandated when engineering and administrative controls cannot reduce exposure to below PEL (NIOSH 1996)
- That PPE can protect workers from hazards is well established (Salazar et al.1999)

Link – Association between PPE Use and Disease Prevention

- PPE Non-use important contributor to occurrence of occupational diseases (Salazar et al 1999)
- Compliance with Universal Precautions (prevention of infectious exposure) found to be low (31% - 38%) among *Physicians* (Michalsen et.al. 1997)
- Due to low perceived risk few workers use hearing protection consistently enough to prevent hearing loss (Lusk, Kerr & Kauffman 1998)

We never thought this could happen!

Determinants of PPE Use

- Individual
  - Training
  - Knowledge of Hazard
  - Perceived Susceptibility
  - Perceived and experienced health effects
  - Perceived effectiveness of PPE
  - Comfort
  - Impact on work performance

(Salazar et al. 1999), (Burstyn & Teschke 1999), (ISEA 2001)

Determinants of PPE Use

- Social/Cultural
  - Organizational culture
  - Co-worker & Supervisor influence
  - Workplace Policies
  - Employer Enforcement
  - Incentives

(Salazar et al. 1999), (Burstyn & Teschke 1999), (ISEA 2001)

Determinants of PPE Use

- Environmental
  - Job Task Requirements
  - Task Duration
  - Worksite ergonomics/construction
  - Weather conditions
  - Availability of PPE

(Salazar et al. 1999), (Burstyn & Teschke 1999), (ISEA 2001)

Health Belief Model Constructs

(Glanz 2002)

- **Perceived Susceptibility**
  - Belief regarding chance of getting a condition
- **Perceived Seriousness**
  - Belief of how severe a condition is and what its consequences are
- **Perceived Benefits**
  - Belief in the effectiveness of the suggested action to reduce risk or impact
- **Perceived Barriers**
  - Tangible and psychological costs of the advised action
- **Cues to Action**
  - Strategies to activate ones readiness to take action
- **Self Efficacy**
  - Ones confidence in ones ability to take action and achieve desired results

All we said was please use PPE!

Health Belief Model Uses in Successful Interventions (Glanz 2002)

- Champion (1993) – Breast Cancer Screening
- Champion (1997) - Mammography Screening Behaviors
- Steers (1996) - AIDS Protective Behaviors

Summary

- Occupational Illness prevention is an important health issue – especially in small shops where majority work but resources are scarce
- Use of PPE can be a critical factor in prevention of Occupational Illnesses
- Interventions targeting behavior change using constructs from the Health Belief Model have been successful

Gaps Based on Review of Literature

- Majority of worksite studies do not have theoretical models guiding their interventions (Ronis et al. 2006)
- Health Belief Model not directly used in worksite studies but is used successfully in many interventions (Glanz 2002)
- HBM- Cues to Action: little is known about relative impact, more study needed (Glanz 2002)
- Very limited studies of PPE use in small shops or organizations (Lazovich et al. 2002)
- No HBM specific instrument developed to apply to workplace studies (Ronis et al. 2006)

Research Undertaken Based on Gaps

- Develop an instrument that assesses HBM constructs for use in industrial workplace settings
  - Focus on industrial shop specific determinants
  - Use focus groups to establish validity
  - Use test-retest group to establish reliability
  - Implications

Research Questions

- Is the developed instrument applicable and relevant to the occupational setting?
- What is the validity and reliability of the developed instrument?
- Does the developed instrument have sufficient validity and reliability to assess the significance of the determinants which predict use of PPE?

Study Limitations

- Study did not take place in a controlled environment under experimental research conditions.
- The sample of participants is small.
- Several self reported behaviors are used in the study including incidence of PPE use, factors influencing use of PPE and perception of cues to action.

Study Delimitations

- Study population was selected using convenience, availability, and experience resulting in a small n.
- Study population was derived from shops where PPE is expected to be frequently employed and where workers are required by OSHA directives to employ PPE.
- Only experienced workers and supervisors were used in the study.

Methodology

Overview

- Develop and Test HBM Based Instrument
  - Base on Champions Health Belief Model Scale
  - Modify for the Occupational Setting
  - Add Self Efficacy Questions based on Stanford Patient Education Research Center Chronic Disease Self Efficacy Scale
  - Test for Validity and Reliability

Quasi-Experimental Design

- Three Groups to establish validity & reliability
- Validity
  - Expert Focus Group (6)
    - Key personnel with expertise on PPE use requirements
    - Establish face and content validity
  - Employee Focus Group (6)
    - Range of employees including supervisory and worker levels
    - Reviewed readability and relevance
- Test-Retest Group
  - Employees from industrial shops requiring PPE
  - Test/Re-test method to establish reliability

Recruitment

- Voluntary
- Expert Focus Group selected by researcher
  - Base upon position and role in PPE monitoring
  - Experienced personnel
- Employee Focus Group selected by Expert Focus Group
- Test-Retest Group
  - Utilize both focus group recommendations
  - Derived from shops where PPE is required
- Notify commanders and key supervisors
  - Presented in commanders staff meeting
  - notify via email
  - Personal contact with each commander
  - Provide researcher contact info
- IRB and Permission from Installation Commander

Focus Group Population

- Expert Focus Group (n=6)
  - Environmental Manager, Former Environmental Manager, Bioenvironmental Engineering Technician (IH), Public Health Technician, Maintenance Squadron Commander (PH and Health Ed. Background), Ground Safety Technician,
  - 2 Judge Content Validity subgroup
    - 2 most experienced personnel from focus group
    - Involved in calculating Content Validity Index (Di Iorio – 2005)
- Employee Focus Group (n=6)
  - Vehicle Maintenance Supervisor, Sheet Metal Shop, Aircraft Maint. QA, Aircraft Crew Chiefs (2), Vehicle Maintenance Line Worker

Test-Retest Group Population

- Test-Retest Group (n=23)
  - Convenience Sample of workers from shops where PPE is required
  - Invited and encouraged to participate from selected shops
  - Selected shops notified by Expert Focus Group members
  - Cross section of industrial shops where hazards include
    - Noise
    - Parts cleaning chemicals & solvents
    - Inhalation hazards from paints and sand blast materials
  - Military only participants
  - Demographics
    - Age range (18-55) Majority over age 35
    - Experience Level (1-20+ years) Majority over 10 years
    - Gender – All Males
    - All employees had PPE training

Procedures
(Focus Group)

- Researcher Developed Draft Instrument
  - Used Champion HBM Scale
  - Used Stanford Chronic Disease Self Efficacy Scale
  - Used construction suggestions from Lusk, Di Iorio, et.al.
- Present to Focus Groups
  - Review instructions, purpose of the study & procedures for group
  - Reviewed and refined the instrument as a group and made changes until consensus was attained
- Present to Content Validity Sub-group
  - Conduct 2 Judge Content Validity Index Procedure (Di Iorio – 2005)

Procedures
(Pilot Group)

- Present to Test, Re-test Group
  - Classroom setting
  - Consent and demographic info collected
  - Trained proctor reviewed instructions & administered survey
  - Presented in paper format
  - Collected in locked box
  - Re-administered in *exact* same way 2 weeks later for re-test

Instrument

- Cover Design considerations
- Intro Letter and detailed instructions
- Refresher instructions on each page
- 5 point Likert scale format

Instrument

- Constructs
  - Questions 1-6 : Perceived Susceptibility
  - Questions 7-13: Perceived Seriousness
  - Questions 14-17: Perceived Benefits
  - Questions 18-25: Perceived Barriers
  - Questions 26-33 : Cues to Action
  - Questions 34-39: Self Efficacy

Analysis

- Test for Validity
  - Focus Group
    - Face Validity (Qualitative)
    - Content Validity (Qualitative and Quantitative)
  - 2 Judge Comparison Content Validity Index
    - Developed CVI form
    - Two most experienced Expert Focus Group members
    - Compared ratio of scales rated "quite relevant" and "very relevant" to total number of scales evaluated
    - Use method from Di Iorio (2005) to calculate

Analysis

- Test for Correlation
- Paired T-test for comparison of sample means
- Cronbach's Alpha for reliability
  - Test/Re-test method at 2 week interval
  - Analyze total and by construct

Results

Face and Content Validity

- Expert Focus Group
  - Qualitative Assessment of applicability and relevance to the setting
  - Revised questions based on experience and knowledge of setting
  - Positive comments on validity based on knowledge and direct experience
  - "This can give us valuable information for future uses, I cant wait to see how it turns out".

Face and Content Validity

- Fleisch-Kinkaid readability = 12.2
- Content Validity Index
  - Two judges from expert focus group
    - Completed CVI form
    - Ratio of quite relevant and very relevant versus total
    - CVI recommended = 0.9 or higher (Di Iorio, 2005)
    - Study CVI calculated at 1.0

Face and Content Validity

- Employee Focus Group
  - Qualitative Assessment of applicability and relevance to the setting
  - Took instrument and gave feedback
  - Recommended only minor changes in wording
  - Favorable review of readability
  - Comment: "Wow, I whish all occupational surveys were written this way."
  - Comment: "This gets to the heart of why we do or don't wear PPE."

Correlation for Groups (Test, Re-test)

- Significant correlation between groups
  - Perceived Susceptibility r=.73, p<.05
  - Perceived Seriousness r=.56, p<.05
  - Perceived Benefits r=.44, p<.05
  - Perceived Barriers r=.67, p<.05
  - Cues to Action r=.80, p<.05
  - Self Efficacy r=.71, p<.05

Paired t-test results

- No significant difference between groups
  - Perceived Susceptibility t(22)=.87, p=.39
  - Perceived Seriousness t(22)=1.05, p=.31
  - Perceived Benefits t(22)=1.03, p=.31
  - Perceived Barriers t(22)=-.65, p=.52
  - Cues to Action t(22)=.55, p=.59
  - Self Efficacy t(22)=.11, p=.92

Reliability – Cronbach's Alpha

- Overall Reliability
  - Test = .314
  - Re test = .612
  - Combined = .706
- When several Constructs measured simultaneously – Low alpha expected (Gliem, 2003)

Construct Reliabilities

- Perceived Susceptibility
  - Test = .655
  - Re test = .596
- Perceived Seriousness
  - Test = .093
  - Re test = .460

Construct Reliabilities

- Perceived Benefits
  - Test = .490
  - Re test = .582
- Perceived Barriers
  - Test = .684
  - Re test = .626

Construct Reliabilities

- Cues to Action
  - Test = .581
  - Re test = .269
- Self Efficacy
  - Test = .523
  - Re test = .740

Reliability After Deletions

- Test = .326
- Retest = .672
- Combined = .727
- Perceived Susceptibility: Test =.770; Retest =.772
- Perceived Seriousness: Test = .405; Retest = .637
- Perceived Benefits: Test = .646; Retest = .665
- Perceived Barriers: Test = .781; Retest = .751
- Cues to Action: Test = .634; Retest = .354
- Self Efficacy: Test = .615; Retest = .750

Discussion

Major Findings

- Validity
  - Assesses determinants of PPE use
  - Effectively uses HBM constructs
  - Very good face validity
  - High Content Validity
  - Bottom Line = Valid instrument for the military small industrial shop setting

Major Findings

- Reliability
  - Variable
  - Construct Dependent
  - Majority of constructs had good reliability
  - Valuable information gained for further study
    - Constructs requiring further refinement
    - Possible methods to effectively target future efforts

Research Question 1

Is the developed instrument applicable and relevant to the occupational setting?

- Focus Group Qualitative and Quantitative Assessment
  - Relevant and Applicable
    - Crucial to acceptance of instrument in the setting
    - Less than 20 minutes to complete
    - Targets the important factors in the setting
  - Targets HBM Constructs and determinants
  - Focus Group Work vital to outcome

Research Question 2

What is the reliability and validity of the developed instrument?

- Validity is well established (CVI = 1.0)
- Reliability lower than hoped
  - Not unexpected for instrument measuring multiple constructs (Gleim, 2003).
  - Lower Cronbach's Alpha in Perceived Seriousness and Cues to Action constructs
  - Important information for future studies
  - Results not discouraging
    - Alpha scores depend in part on context of the study
    - In context, alpha scores could be adequate
    - Sufficient reliability for the setting and context

Research Question 3

Does the instrument have sufficient validity & reliability to assess the significance of the determinants that influence PPE use?

- Sufficiently addresses determinants for PPE use
  - Unique instrument with sufficient validity
    - Addresses Determinants
    - Addresses HBM Constructs
- Utility in assessing interventions using HBM constructs
  - Important since majority of studies do not use theoretical models to guide interventions (Ronis, 2006)
  - Includes self efficacy – Not in original Champion HBM Scale (Champion, 1993)
  - Includes cues to action which are not well studied (Janz, 2002)
  - More research needed in this area but this study can serve as a foundation

Recommendations

1. Further assess validity
   a. Add judges to CVI process
   b. Recommend adding outside judge for perspective
2. Consider other tests for reliability
   a. Split half can have utility
   b. Obtain larger sample size at outset

Recommendations

3. **Consider timing of re-test**
   a. Extend time to retest out to 30 days
   b. Lessen chance of subject response recollection
   c. Minimize influence of outside discussions
4. **Employ methods to improve reliability**
   a. Add subjects to increase N
   b. Add more scales to constructs with low alpha

Recommendations

5. Conduct survey at a larger military facility
   a. Reduce bias from close interpersonal working relationships
   b. Reduce discussion of instrument between test and re-test
6. Limit instrument to fewer/single construct
   a. Test each construct of HBM individually
   b. Could result in subject fatigue
   c. In the proper setting could have some utility

Implications for Further Research

- Repeat study at larger military facility
  - Larger Subject Pool
  - Less impact from organizational culture
- Expand Study to Non-military Setting
  - Validity and reliability in a variety of settings
  - Could improve utility or versatility
- Adapt items to study Setting
  - Continue to refine instrument
  - Tailor to specific setting

Implications for Further Research

- Employ Instrument
  - Use refinement procedures for the setting
  - Ultimate goal is to develop an instrument that allows Health Educators to identify determinants that can be used to develop an intervention to promote PPE use
  - Focus on cues to action component

We can tackle this!

Conclusion

- Interventions employing theory driven models are scarce in occupational setting
- No instrument developed to guide HBM based interventions in occupational setting
- Study instrument begins to fill this need
- More work is needed to refine instrument for reliability but key information was gained

Conclusion

- Dedicating efforts to long term worker behavior change is crucial
- Unique opportunity for Health Education
  - Prevention based perspective invokes a new niche for Health Educators
  - Partner with related disciplines such as IH, PH, and Occupational Health Nursing
  - Use Health Education models to drive interventions

Conclusion

- Instrument provides a tool to investigate this area and can open a body of research
- Further research must concentrate on the "shop floor" level of prevention to protect workers from adverse health effects
- Clearly Health Education can provide a vital link in a multi discipline approach to improving worker health

Questions and Answers

Your Feedback Please

Nucleic Acid and Protein Detection Technology: Limitations, Milestones, and the Continuous Search for the Holy Grail

United States Air Force School of Aerospace Medicine (USAFSAM)/PHT, Brooks City Base, TX

Clarise R. Starr, PhD

Successful detection of pathogens and toxins in a deployed situation is only as good as the technology that is implemented. The field of nucleic acid and protein detection is evolving, with transformation to smaller instrumentation with greater computing power that can provide more information faster in a single assay. However, by the time one instrument has been evaluated for potential use, it is quite common for another generation of technology to be released. The current topic will focus on trends in this field, and discuss currently fielded instrumentation for nucleic acid and protein detection utilized by the USAF and the DoD. Our evaluations of Film Array and Meso Scale PR2, two new instruments that are thought to be the next generation of nucleic acid and protein detection, respectively, will be presented. In addition, the journey to generating a single platform for both pathogen and toxin detection will be discussed with emphasis on current and future technologies that may eliminate the foreknowledge needed to design target specific assays.

U.S. AIR FORCE

Nucleic Acid and Protein Detection Technology: Limitations, Milestones, and the Continuous Search for the Holy Grail

Clarise Rivera Starr, Ph.D.
Molecular Biologist
USAFSAM/PHT
Brooks City-Base, TX

Every Airman a Force Multiplier
August 2010 AFMS Research Symposium

Polymerase chain reaction (PCR) is a rapid way to detect and amplify DNA sequences

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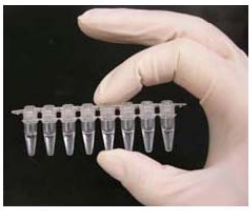

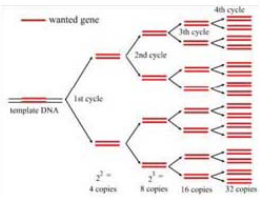

- Very sensitive (~1 copy)
- Fast (1-2 hours)
- Specific (probe dependent)
- Potential for high-throughput

 2

The Limitations of PCR

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- Requires specialized reagents/equipment
- Target detection only as good as the purified sample
- High-throughput depends on technology
- Amplicon contamination potential
- Must have fore-knowledge of target testing
- Multiplexing limitations
- Mutation and effect of gene/assay targets

 3

JBAIDS/Dry Down Assays

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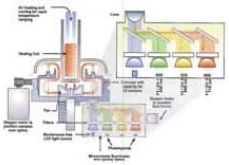

<u>32-well Carousel</u>

NC x 2

PC x 2

6 Samples X 2

IC (6) x 2

EC x 2

 4

JBAIDS Duplexing Using Pulsar 650

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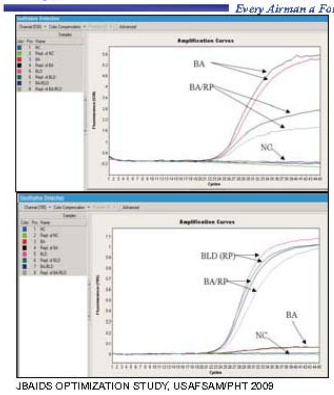

Channel 1: BA pos control

BA + IC

Channel 3: IC pos control

BA + IC

JBAIDS OPTIMIZATION STUDY, USAFSAM/PHT 2009

Idaho Technology Film Array (Multiplexing)

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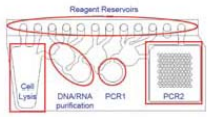
Easy to use

No pre-processing needed*

20 targets 1 sample

Internal controls included

1 sample takes over 1 hour to complete



Images from Idaho Technology Product Literature

Film Array Data: Clinical

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"Negative" Nasal Wash/NP Swab Samples (n=25)

RESULTS	ORGANISMS	ASSAY
Positive (52%)	Bordetella pertussis	Bp
	C. pneumoniae	Cpne
	Coronavirus	229E, OC43
	Enterovirus	Entero 1, 2,
	Human Rhinovirus	HRV1, HRV2, HRV3, HRV4
	Influenza A	FluA pan2
	Parainfluenza Virus	PIV2
Negative (48%)		

Adeno Nasal Wash Samples (n=25)

RESULTS	ORGANISMS	ASSAY
Positive (64%)	Adenovirus	adeno
Co-infection (28%)	C. pneumoniae	Cpne
	Coronavirus	229E, OC43
	Enterovirus	Entero 1
	Human Metapneumovirus	hMPV
	Human Rhinovirus	HRV1, HRV2, HRV3, HRV4
	Influenza A	FluA H1
	Parainfluenza Virus	PIV2, PIV4
	Respiratory Syncytial Virus	RSV
Negative (8%)		

Influenza A Samples (n=25)

RESULTS	ORGANISMS	ASSAY
Positive (56%)	Influenza A	FluA pan1, pan2; FluA H1, FluA H1 09, FluA H3
Co-infection (44%)	Bocavirus	Boca
	Bordetella pertussis	Bp
	Coronavirus	OC43
	Enterovirus	Entero 1
	Human Rhinovirus	HRV1, HRV2, HRV3, HRV4
	Influenza B	FluB

Parainfluenza Samples (n=24)

RESULTS	ORGANISMS	ASSAY
Positive (79%)	Parainfluenza Virus I and III	PIV1, PIV3
Co-infection (22%)	Bocavirus	Boca
	C. pneumoniae	Cpne
	Human Rhinovirus	HRV4
	Enterovirus	Entero 1

H1N1 2009 Samples (n=25)

RESULTS	ORGANISMS	ASSAY
Positive (44%)	Influenza A	FluA-pan1, pan2; FluA-H1, FluA-H1-09
Co-infection (52%)	Bocavirus	Boca
	C. pneumoniae	Cpne
	Coronavirus	229E, HKU1, OC43
	Enterovirus	Entero 1
	Human Metapneumovirus	hMPV
	Human Rhinovirus	HRV1, HRV2, HRV3, HRV4
Negative (4%)		

Respiratory Syncytial Samples (n=25)

RESULTS	ORGANISMS	ASSAY
Positive 48%	Respiratory Syncytial Virus	RSV
Positive (4%)	Human Rhinovirus	HRV2, HRV3, HRV4
Co-infection (44%)	Bocavirus	Boca
	Coronavirus	HKU1
	Enterovirus	Entero 1, 2
	Human Rhinovirus	HRV1, HRV2, HRV3, HRV4
	Parainfluenza Virus	PIV2
Negative (4%)		

Film Array Data: Environmental

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Environmental (41 Samples)		
RESULTS	**ORGANISMS**	**ASSAY**
Positive (44.2%)	Adenovirus	adeno
	Human Rhinovirus	HRV1, HRV2, HRV3
	Coronavirus	HKU1
	Human Rhinovirus	HRV1, HRV2, HRV3
	Parainfluenza Virus	PIV3
Negative (55.8%)		

Luminex Assay (Multiplexing)

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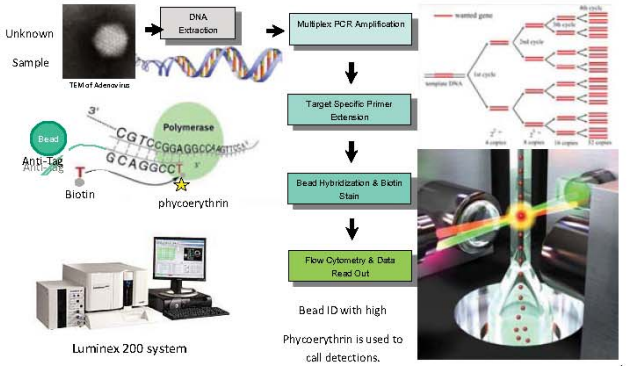

Isothermal Amplification

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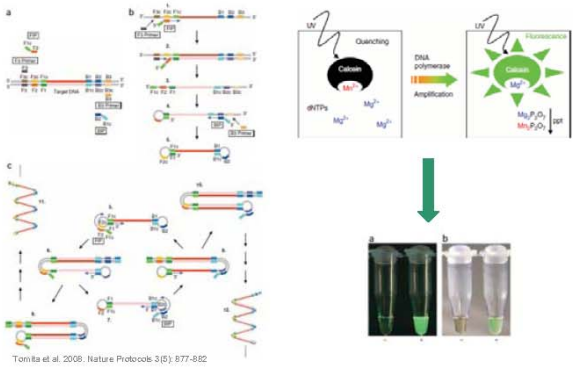

Tomita et al. 2008; Nature Protocols 3(5): 877-882

Pyrosequencing

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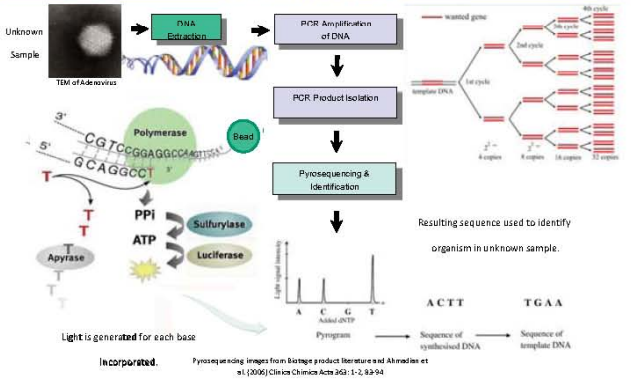

Pyrosequencing images from Biotage product literature and Ahmadian et al. (2006) Clinica Chimica Acta 363: 1-2, 83-94

Summary

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- Nucleic acid detection technology has evolved but has brought with it new challenges.
- Great care needs to be taken to fully understand the limits of any new technology, and always keep in mind how a new technology can translate into a <u>useful</u> tool to meet mission requirements.
- Bigger doesn't necessarily mean better. More expensive doesn't necessarily mean better (Location, Location, Location!).
- Always keep in mind the sample that you are testing and what unique challenges come with it.

Immunoassay Overview

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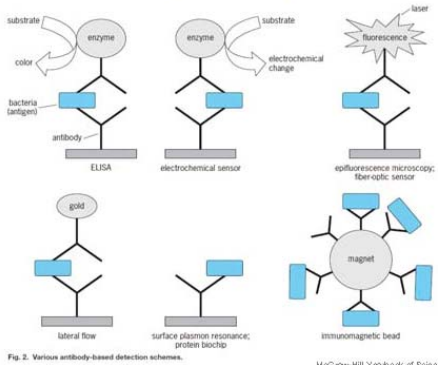

Fig. 2. Various antibody-based detection schemes.

McGraw-Hill Yearbook of Science & Technology. 2003

Limitations to Protein Detection

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- Only as good as the antibody! (Pure protein versus organisms)
- Only as good as the antibody! (Monoclonal vs. Polyclonal)
- Only as good as the antibody! (Commercially available versus starting from scratch–$$$)
- Sensitivity/specificity decreases in complex matrices.
- Viral targets difficult to detect (within clinically relevant sensitivities).
- What is a positive? Establishing what is background "noise" can be challenging and depends on matrix and system used to detect the protein.

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BioVeris M1M

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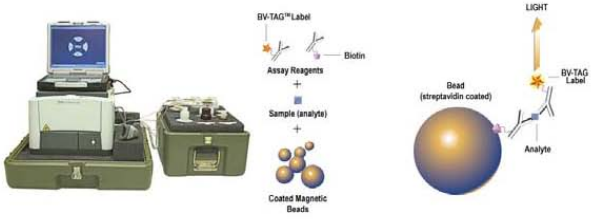

15

Meso Scale 1800/1900 PR2

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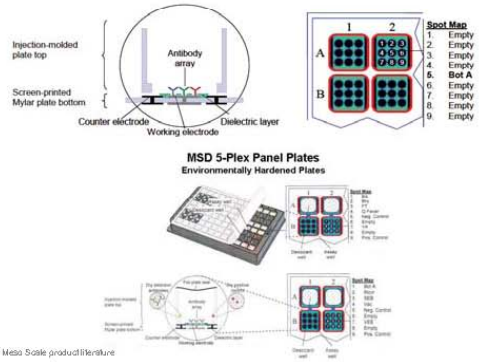

Images from Meso Scale product literature

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Meso Scale Data

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	1800	1900	M1M
SEB	5 pg/ml	5 pg/ml	1000
Cbot	50 pg/ml	50 pg/ml	100
BG spores-live	30 spores	30 spores	no assay
BG spores-inactive	12,000 spores	TBD	no assay
BG veg	90,000 org	TBD	no assay
oval	5 pg/ml	5 pg/ml	no assay
MS2	10000 org	10,000 org	no assay
EHEC	90 org	TBD	90,000 org
Shigella	9,000 org	TBD	900,000 org
Flu A (H1N1)	1581 $CEID_{50}$/ml	1581 $CEID_{50}$/ml	no assay
Flu B	0.28 $CEID_{50}$/ml	0.28 $CEID_{50}$/ml	no assay
Adv 14	225 $TCID_{50}$/ml	225 $TCID_{50}$/ml	no assay

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The Holy Grail

Every Airman a Force Multiplier

DARPATECH, August 2005

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Challenges for the Holy Grail

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- Sample processing (DNA vs. Protein)
- Sensitivity (Ab vs. nucleic acid)
- Specificity (Ab vs. nucleic acid)
- Testing in complex matrices
- False positives, scary. False negatives, even scarier!
- Hands-on versus complete automation
- Time, time, time

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Immuno-PCR (I-PCR) and BEADS

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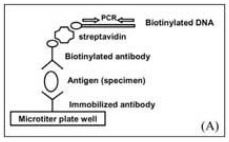

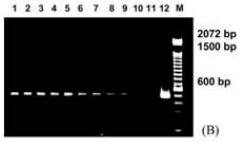

Huang and Chiang. 2004. Clin Chem. 50(9): 1673-1674

Biodetection Enabling Analyte Delivery System (BEADS) Technology

Ozanich, PNNL 2009

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Abscription/HISSS

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Handheld Isothermal Silver Standard Sensor (HISSS)

DARPATECH, Aug 2005

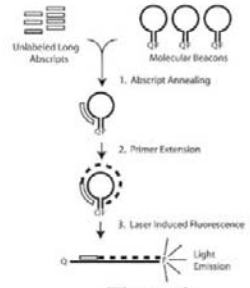

Figure 3

Hanna and McCarthy, 2006, US. Pat # US2010015822A1

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Biosensors/Microcantilevers

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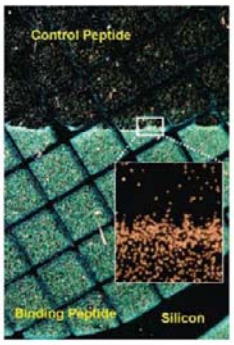
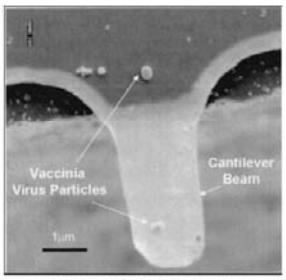


Dhayal et al. 2006. JACS. 128:3716-3721



Gupta et al. (2006). PNAS 103 (36): 13362-13367

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Aptamers

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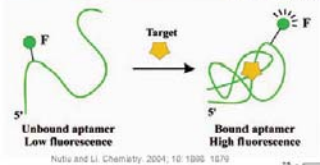
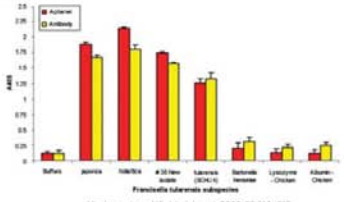


Nutiu and Li. Chemistry. 2004; 10: 1868-1879



Vivekananda and Kiel. Lab Invest. 2006; 86:610-618

Distribution Statement A: Approved for public release; distribution is unlimited. 311 ABG/PA No. 10-305, 5 AUG 10

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Way Forward

Every Airman a Force Multiplier

- New technology vs. old technology
- Overcome sensitivity/specificity issues
- Fast AND correct
- Research developed and kept in DoD
- Utility in nonprofit Government-sponsored labs

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Conclusions

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Holy Grail may not be feasible with current technologies, but it is vital to keep up with emerging technology developments and determine if it can be modified to help bring the end product we need.

Acknowledgments

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Upper Respiratory Virus Serotype Panel for the Pyrosequencer

Applied Technology Center

James Baldwin, DR02/Molecular Biologist

Pyrosequencing is an excellent way to detect clinical infections. Unlike PCR-only assays, the pyrosequencer can discriminate sequences based on DNA sequence. Sequence evaluation allows precise detection while often providing the serotype of the detected organisms. Under investigation is the use of deeply multiplexed PCR tests followed by a rapid pyrosequencing step to identify detected organisms. The test uses low-specificity primers to amplify related sections of important upper respiratory virus genomes. The exact identity of any given product is then determined by DNA sequencing short sections of the PCR product (up to 50 bp). The sequences are used as tags to accurately identify organisms from a database of possible results extracted from Genbank. Assays were designed for the pyrosequencer to detect and serotype influenza (A, B, C), several human coronaviruses (HKU1, NL63, 229E, OC43, SARS), adenovirus (most serotypes including 3, 4, 7, 11, 14, 21), parainfluenza (1, 2, 3, 4), metapneumovirus, Picornaviridae (most rhinovirus, enterovirus, Coxsackie virus, echovirus, and poliovirus), and respiratory syncytial virus (A, B). The assays were designed to work in a single multiplexed tube and as individual tests. Results indicate that adenovirus, coronavirus, and Picornaviridae assays offer a robust detection and identification method. In some assays (Picornaviridae, for example) we can theoretically detect/serotype over 100 viruses in a single test by sequencing less than 25 bp. This work is designed to support the public health mission of the DOD through enhanced diagnostic testing of clinical samples, including those from the recruit and enlisted populations.

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Upper Respiratory Virus Serotype Panel for the Pyrosequencer

AFMS Research Symposium
August 25, 2010

James C. Baldwin, Ph.D.
USAF Civilian Molecular Biologist
Applied Technology Center
2484 Gillingham Drive, B-175W
Brooks City-Base, TX 78235

U.S. AIR FORCE

Disclaimer

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The use of trade name(s) in this presentation does not constitute an endorsement or approval of the use of such commercial hardware or software but rather serves to examine the scientific merit and potential uses of the technology. This presentation may not be cited for purposes of advertisement.

Upper Respiratory Viruses Cause Substantial Morbidity and Mortality

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- *Over 52 strains of human adenovirus.*
- *Over 10 major strains of influenza.*
- *5 strains of noteworthy Coronavirus.*
- *Over 15 major classes of human pathogens in Picornaviridae.*
- *Most of these are not clinically relevant.*
  - *Infrequently seen*
  - *Cause illness of limited severity*
- *However, several strains are of the highest concern.*
  - *These viral serotypes can impact the health and readiness of military personnel*

Polymerase chain reaction is a rapid way to detect and amplify DNA sequences.

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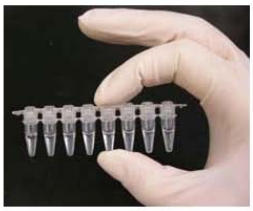

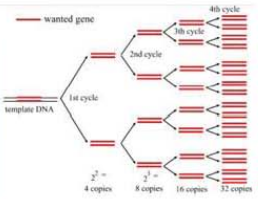

- *Amplification can be highly specific*
- *Can detect as little as a single base difference*

What Is Desirable in a PCR Assay?

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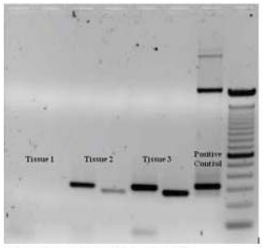
- Required specificity (or non-specificity)
- Low complexity of sample handling
- Few unexpected catches
- High throughput
- Low cost
- Future proof



PCR EtBr Gel image, Roland Gaschain

5

Stereotyping Detection Assays

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- Bioinformatics analysis was used to determine the best genome sites for placing PCR test.
  - The analysis identified regions of similarity and divergence
  - Primers (some degenerate) with the best thermodynamic properties in these regions were selected
  - The divergent regions were sequenced
  - The DNA sequence is usable as a barcode

6

Designing the Assays

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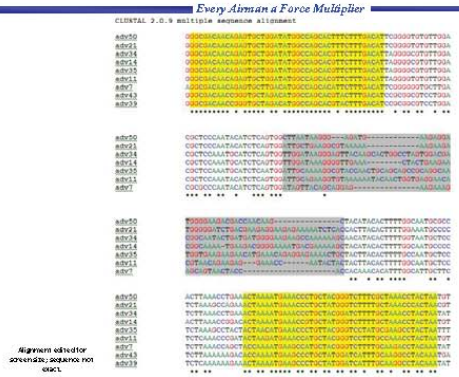

7

PyroMark Q96 Platform

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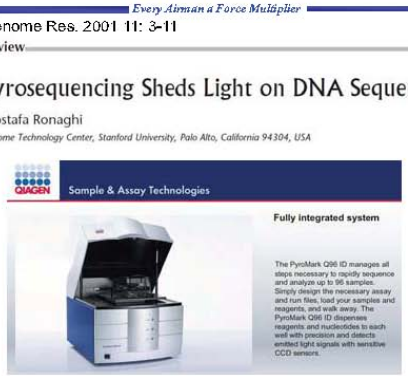

Genome Res. 2001 11: 3-11

Review

Pyrosequencing Sheds Light on DNA Sequencing

Mostafa Ronaghi

Genome Technology Center, Stanford University, Palo Alto, California 94304, USA

QIAGEN Sample & Assay Technologies

Fully integrated system

The PyroMark Q96 ID manages all steps necessary to rapidly sequence and analyze up to 96 samples. Simply design the necessary assay and run files, load your samples and reagents, and walk away. The PyroMark Q96 ID dispenses reagents and nucleotides to each well with precision and detects emitted light signals with sensitive CCD sensors.

8

Pyrosequencing

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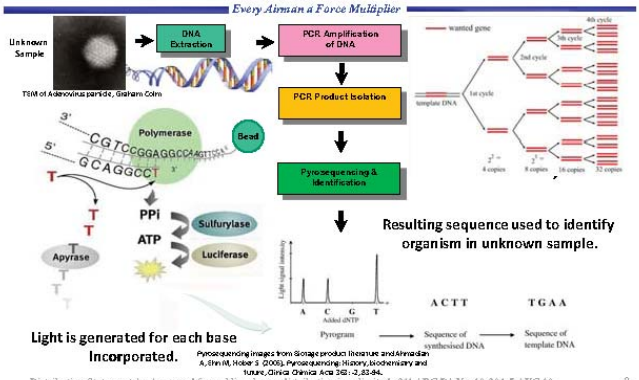

PCR-Based Virus Detection and Pyrosequencing

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- *This proof of concept evaluation focused on deeply multiplexed PCR tests*
  - *Each test must detect and serotype the clinically relevant strains*
  - *Test will leverage the PyroMark Q96 system to read out results of the PCR for deep multiplexed detection*
  - *Each assay will be usable in other pyrosequencers or as a gel/RT-PCR reaction (without the sequence) for future proofing*

Planned Virus Detection

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- *Under investigation are multiplexed tests to detect and serotype 7 major classes of virus:*
  - *Coronavirus*
  - *Human adenovirus*
  - *Influenza virus*
  - *Metapneumovirus*
  - *Parainfluenza*
  - *Picornaviridae*
  - *Respiratory syncytial virus*
- *The project was accomplished as 10 individual PCR tests*

Coronavirus Assay

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- *This test is a single PCR reaction theoretically able to detect most known Coronavirus that affect humans and animals*
- *It can discriminate 50 unique strains in Genbank*
- *It has been certified in wet lab to detect:*
  - *SARS* (via synthetic DNA stimulant)*
  - *HKU1**
  - *OC43*
  - *229E*
  - *NL63**

Human Adenovirus

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- This test is a single PCR reaction theoretically able to detect most adenovirus strains affecting humans and animals
- It can discriminate 52 unique strains in Genbank
- It has been certified in wet lab to detect Human Adenovirus 3,4,7,11,14, and 21

Influenza A&B&C Virus

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- This test is composed of 3 PCR reactions (one for each of Influenza A, B, & C. It is theoretically able to detect most strains affecting humans and animals
- The test cannot definitively describe N and H types in Influenza A as the PB1 segment is sequenced
  - However, results are strongly correlated with certain N H groups, with over 95% correlation in most cases
- A assay can discriminate 1275, B 50, and C 33 unique strains as observed in Genbank
- It has been certified in wet lab to detect clinical samples of Influenza A (N1H1), B, and C

Metapneumovirus

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- This test is a single PCR reaction. It will detect all strains of Human Metapneumovirus in Genbank
- The assay can discriminate 13 unique strains as observed in Genbank
- It has been certified in wet lab to detect clinical samples of Metapneumovirus

Parainfluenza 1&2&3

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- This test is composed of 2 PCR reactions (one for each 1 & 3 and another for 2). It is theoretically able to detect most strains affecting humans and many affecting animals
- The 1&3 assay can discriminate 15 and the 2 assay 10 unique strains as observed in Genbank
- It has been certified in wet lab to detect clinical samples of Parainfluenza
- The assay will also detect the Sendai virus and mumps virus due to their similarity

Picornaviridae

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- This test is composed of a single PCR reaction. It is theoretically able to detect most strains of this entire family of virus by sequencing the ribosomal insertion site
- The assay will detect and discriminate 963 unique strains of the following important groups of virus:
  - Coxsackievirus
  - Echovirus
  - Enterovirus
  - Poliovirus
  - Rhinovirus

Respiratory Syncytial Virus

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- This test is composed of a single PCR reaction. It is theoretically able to detect most strains of Respiratory Syncytial Virus from humans
- The assay will detect and discriminate 12 unique strains as observed in Genbank
- It has been certified in wet lab to detect clinical samples of Respiratory Syncytial Virus

After PCR Samples Are Purified and Sequenced

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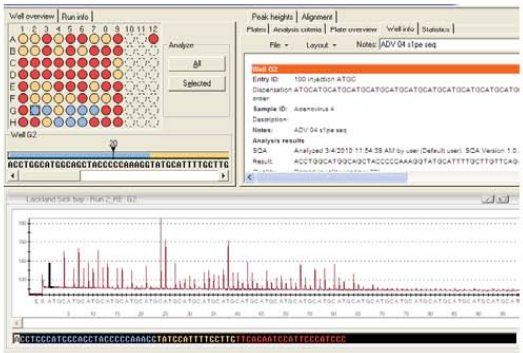

How the Results Are Determined

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- Detection is made by simply matching the resulting sequence to a database of possible hits.
- By crafting the database we can display as many or as few hits as desired by the end user.

```
Selected Representative Database:
gi|89515561 15330-15624 Human coronavirus HKU1   ACTGCTAGTACCACCAGGCTTAACATAATAGCAACCGCCACACATAACTATTTCAC
gi|38018022 15080-15374 Human coronavirus OC43   ACTACTAGTGCCACCAGGCTTAACATAATAACAGCCACCACACATAACAATTTCAC
gi|49169782 14175-14469 Human coronavirus NL63   ACAAGTCGTACCACCTGGCTTAAAATAAAACCACACATTAGAATAAACAACTTCTG
gi|12175745 14256-14550 Human coronavirus 229E   ACAAGTTGTACCACCAGGTTTAAAATAAAACCCACCATTTGAATAAACAACCTCGG
gi|30271926 15146-15440 SARS coronavirus         GCATGATGTTCCACCTGGTTTAACATATAGTGAGCCCCACACATGACCACTCTCAC
gi|71796704 15108-15402 bat SARS coronavirus     GCATGATGTCCCACCTGGTTTCACATATAATGAGCCCCACACATGACCACTCTCAC
gi|17523670 15080-15374 Bovine coronavirus       ACTACTAGTGCCACCAGGCTTAACATAATAACAGCCACCACACATAACAATTTCAC
gi|158632126 14132-14426Turkey coronavirus       ACTGCTAGTGCCACCAGGTTTTACATAAATACCACCTGTAGCTAAAACAGTTTCAG
gi|212691378 13157-13448Thrush coronavirus       ACTGCTAGTGCCACCTGGTTTTAACATATAGATTATTACCAGACACCACAACTTCTG
gi|19385576 14256-14550 Porcine epidemic diarrh ACAGCTAGTACCACCTGGCTTCAAATAAAACCTCCATTAGAATAAACAACCTCAG
gi|85718614 15080-15374 Porcine hemagglutinatin ACTACTAGTGCCACCAGGCTTAACATAATAACAGCCACCACACATAACAATTTCAG
gi|146411511 555-846    Asian leopard cat coron ACTGCTAGTGCCACCTGGTTTTACATAGAGGTTGTTACCAGACACTACCACTTCTG
gi|156910077 14085-14379Infectious bronchitis    ACTACTAGTGCCACCAGGCTTAACATAAATGCCACCTGTAGCTAAGACAGTTTCAG
```

Results Can Be Determined with the Provided Software

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- *The assays work quite well on extracted clinical samples.*
- *Using these assays we have observed previously undetected infections and serotyped known ones.*

2010-07-29

IdentiFire
Summary Report

Biotage

Sample Id	Result	Score	
CCH 4	gi|83839241:1-344 Antwerp rhinovirus 98/99 isolate	63.4	Details
CCH 5	No hits found.	0	Details
CCH 9	gi|38347786:380-500 Human coxsackievirus A10	76.5	Details
CCH 10	gi|147868570:367-486 Human rhinovirus sp. isolate	100	Details
CCH 11	RV051_gi|217316402|gb|FJ445136	92.5	Details
CCH 4	No hits found.	0	Details
CCH 5	RV028_gi|95102531|gb|DQ473508.	100	Details
CCH 8	No hits found.	0	Details
CCH 10	gi|147868570:367-486 Human rhinovirus sp. isolate	93.8	Details
CCH 11	RV051_gi|217316402|gb|FJ445136	92.5	Details
CCH 4	No hits found.	0	Details
CCH 5	No hits found.	0	Details

The IdentiFire Software Produces Fair Results

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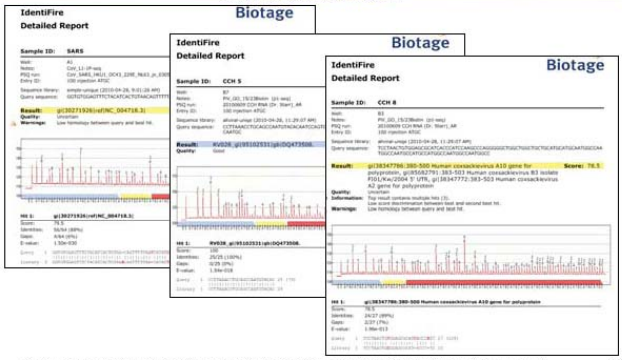

Detection of Novel Viruses Can Be Done with Public Tools

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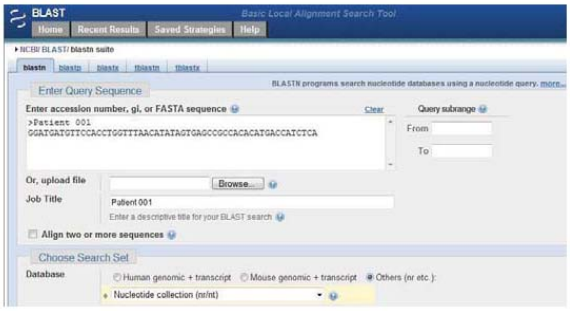

Detection of Novel Viruses Can Be Done with Public Tools

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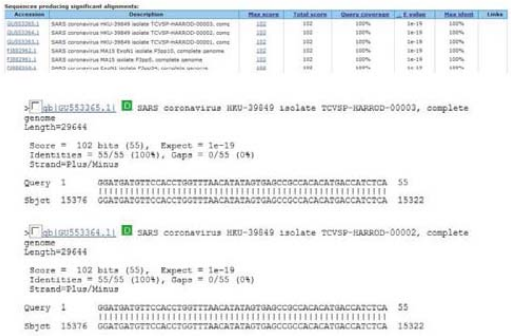

Typical Limits of Detection

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- Most samples are readily detected down to 10^{-7} or 10^{-8} dilution from ATCC stocks.
- This level of PCR detection indicates an approximate detection range of 100 $TCID_{(50)}$ per mL based on stated titers of the original ATCC stocks. This level is fairly consistent across most of the assays tested.
- Due to the nature of pyrosequencing, it is very hard (almost impossible) to get a false positive.
- However, false negatives are fairly common (1-10%). This is manageable by using replicates.

Progress

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- Currently we have our stated minimum deliverables working.
  - We have determined that despite several limitations, the PyroMark Q96 instrument offers a low cost platform for the rapid detection and serotyping of clinically relevant viral diseases.
  - We can easily detect and serotype over 20 clinically relevant virus targets.
- We implemented 7 important tests that with further development could be used in future clinical tests or in disease surveillance.

Conclusions

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- The proof of concept evaluation will be done shortly.
  - Proof of detection backed by firm numbers from large groups of clinical unknowns remains to be completed (likely in year 2 of the project).
  - Synthetic controls and dilutions and small lots of clinical samples show sufficient detection sensitivity.
  - Pyrosequencing detection software is somewhat lacking but serviceable.

Future Directions

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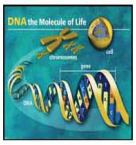
- The degenerate PCR methodology offers several unique directions for future assays.
  - Allows deep multiplexing.
  - Is amenable to several different instruments.
  - Can readily detect large subsets of similar organisms.
  - Can individually identify members of these groups.

PHT Mission

Every Airman a Force Multiplier

PHT Mission:

Provide continual and rapid evaluation, validation, and transition assistance of new off-the-shelf technologies and identify emerging technologies ("technology discovery") to fill critical gaps in force protection, rapid diagnostics, epidemiology, and preventive medicine, including CBRNE identification, to meet Air Force global mission requirements



Acknowledgments

Every Airman a Force Multiplier

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- *David L. Maserang, Ph.D.*

Project Management:
- *MSgt George Viale**
- *Elizabeth Escamilla*

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- *Jeffery Smith*
- *Roel Escobar*

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- *Michael Nedrick*

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Upper Respiratory Virus Serotype Panel for the Pyrosequencer

AFMS Research Symposium
August 25, 2010

James C. Baldwin, Ph.D.
USAF Civilian Molecular Biologist
Applied Technology Center
2484 Gillingham Drive, B-175W
Brooks City-Base, TX 78235

U.S. AIR FORCE

The Use of Retinal Photographs for AFSOC Flyers at Risk for Laser Eye Injuries: Evaluation as Screening Exam

United States Air Force School of Aerospace Medicine (USAFSAM)/FEER

Lt Col/Dr. Christopher Hudson

The increasing availability of lasers has led to a proliferation of laser illuminations of airplane cockpits and crews during flight. Such illuminations not only present flight safety issues such as distraction or visual disturbance, they could in some cases result in damage to the retina. The United States Air Force Special Operations Command (AFSOC) has initiated retinal photographs for its aircrew to be used in the event of a laser eye exposure incident. Since 2004, all flyers were required to have a reference retinal photograph to use as comparison to their exam after a laser exposure. The intent of the retinal photograph is to enhance the ability of the examining provider to detect laser associated retinal damage. The frequency at which such retinal photographs should be repeated as a screening exam has not been determined. The purpose of this study is to examine retinal photographs of AFSOC flyers as a screening exam. As with any screening exam, the factors to consider are the incidence and prevalence of the condition, the sensitivity and specificity of the screening exam, the cost effectiveness of screening, the appropriate screening interval and any unintended consequences related to screening. After considering each of these factors, our recommendation is that baseline retinal photographs for AFSOC flyers are not recommended as an effective as a part of a medical surveillance screening program for potential laser eye injuries. This recommendation is based on the lack of injuries reported in aviators, the limited number of documented ACS injuries over the past 15 years, the cost and lost work time associated with screening and the limited use as a screening tool.

THE EFFECTIVENESS OF RETINAL PHOTOGRAPHS FOR AFSOC FLYERS AT RISK FOR LASER EYE INJURIES: EVALUATION AS SCREENING EXAM



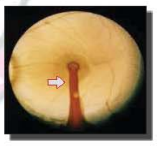
Christopher Hudson, LtCol, USAF, MC, FS
Chief, Aerospace Medicine
377th Medical Group, Kirtland AFB, NM
25 August 2010

DISCLOSURES

- NOTHING TO DISCLOSE

BACKGROUND

- Increasing availability and usage of lasers, both military and civilian
  - Proliferation of laser illuminations of cockpits
  - Flight safety issues-- distraction or visual disturbance
  - Greatest concern- potential damage to the retina
- AFSOC initiated baseline retinal photographs for all aircrew in 2004


RETINAL HEMORRHAGE

3

PURPOSE

- Examine the effectiveness of obtaining baseline retinal photographs for AFSOC flyers as a screening exam for future laser eye injuries
- Apply all factors related to determining the appropriateness as a medical screening exam
- Make overall recommendation to AFSOC/SG regarding baseline retinal photograph policy for AFSOC aviators

BACKGROUND- LASER CLASSIFICATION

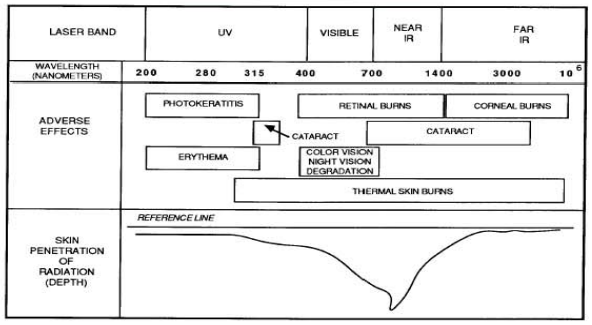
- LASER "Light Amplification by Stimulated Emission of Radiation"
  - Visible and invisible spectrum, from near Infrared to Ultraviolet
  - Powerful lasers available to public
- Classified by ability to cause damage to the eye or skin
  - Class 1 -- incapable of producing damaging radiation

Aircraft-Mounted Targeting Turret Pod

Weapons-Mounted Pointer/ Illuminator/ Target Designator

5

SPECTRUM OF LASERS AND ASSOCIATED INJURIES



LASER CLASSES CONT.

- Class 2 and 3A lasers
  - Most commercially available lasers- laser pointers, infrared thermometers and distance indicators
  - Only purposeful fixation on beam for prolonged time will cause injury
  - Can cause a momentary visual disturbance (after image) or temporary flash blindness
  - In cockpit, presents distraction/safety issue
- Class 3B- Can cause retinal injury
  - Ground-based target designators and non-lethal deterrent devices (dazzlers)
  - Risk of laser eye injuries from both friendly and hostile forces
- Class 4 -- hazard to the eye and skin, diffuse reflection, fire hazard
  - Aircraft based target designators, electo-optical countermeasures

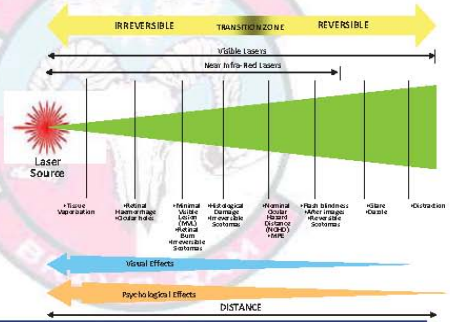
Target Illuminator

7

RANGE OF LASER BIOEFFECTS

Factors which determine severity and duration of ocular symptoms

- Type of laser
- Distance from the source
- Presence of an Ocular device between the eye and laser
- Wavelength



8

LASER EYE INJURY SYMPTOMS

- True retinal laser injury
  - Rapid, painless decreased vision, usually monocular
- Typical ocular symptoms associated with laser illumination of the cockpit (no retinal injury)
  - After image, flash blindness, ocular irritation
  - Binocular symptoms
  - Pain secondary to corneal abrasion from rubbing the eye

MITIGATION OF OCULAR INJURY

- Look away if practical
- Maneuver aircraft to avoid beam
- Shield eyes
- Regular spectacles, sunglasses or visors are not protective
- NVG's only protect a limited field of vision
- Laser Eye Protection (LEP's)
  - Wavelength specific
  - Useful around known laser sources -- friendly fire
  - Limited in hostile settings

10

POLICY

- ANSI Z136.1-2007
  - Surveillance IAW American Nat'l Standard for Safe Use of Lasers
- DoDI 6055.15 --each service shall establish a laser protection program
  - Prevent, identify and track laser related eye injuries
  - Establishes Tri-Service Laser Injury Hotline
- USAF Laser Radiation Protection Program AFOSH 48-139
  - Medical surveillance required for laser workers
  - Outlines steps for suspected laser exposure
- USAFSAM Laser Injury Guidebook
  - evaluation, treatment, reporting of suspected laser eye injuries

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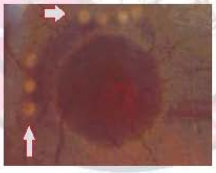
AFSOC POLICY

- AFSOCI 48-1391, dated 2004
  - All flyers and Special Operators (PJ's, CCT, TACP)
  - Modified April 2009 --specific flyers at risk for laser eye injury
    - Based on possible exposure to specific classes of lasers
    - Purpose--baseline retinal photo –compare suspected exposure



RETINAL BURNS

12

METHODS- DATA SOURCES

- Civilian laser incidents – FAA
- Military laser incidents
  - Tri-service Laser Injury hotline
  - Aeromedical Consult Service
  - NASIC
  - AFRL/HEDO
  - AFSC
  - Cost data -- Hurlburt Field Optometry Clinic

HUD GLARE

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RESULTS - MILITARY DATA

- Laser Illumination Events
  - Tri-service Laser injury hotline 2009 data (184 reports)
    - 14% (26/184) of the incidents involved aviators, all reported as hostile action
    - No associated permanent injury in any aviator
  - Ground based events
    - 86% of all reported events
    - 6% (10/158) associated with possible injuries
    - Majority of ground troops exposed by friendly forces
    - Inappropriate use of dazzlers/target designators

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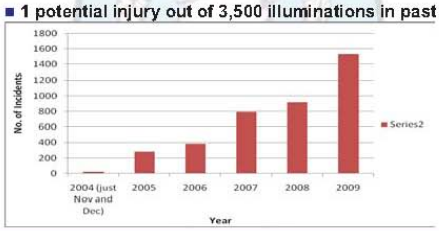
MILITARY DATA CONTINUED

- ACS- reported a single case of an aviator with a potential laser related eye injury in the past 15 years
- NASIC (National Air and Space Intelligence Center)- No reported permanent injuries to flyers
- AFRL/HEDO- 277 reported exposures in 2009, three in aviators, no injuries
- AFSC- AFSAS contains no reports of laser eye injuries in aviators
- Hurlburt Optometry Clinic- multiple evals for suspected injuries, no confirmed cases

RESULTS – FAA DATA

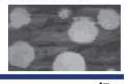
- Increasing number of illumination events each year
  - Over 3500 incidents in past five years
  - Over 1,500 incidents noted in 2009
  - 1 potential injury out of 3,500 illuminations in past 5 years



AFSOC RECORD REVIEW

- Approximately 2830 exams performed since 2004 at the Hurlburt
- One year record review was performed to determine the pathology detected with these photos

- Of the 344 aviator records that were reviewed:
  - 75% (251/344) were normal
  - 22% (74/334) were benign findings or "false positives" such as Congenital Hypertrophy of the Retinal Pigment Epithelium (CHRPE) and Nevus
  - 3% (9/334) were abnormal findings or "true positives" such as Lattice degeneration, suspect glaucoma , macular drusen, optic nerve drusen and retinal holes



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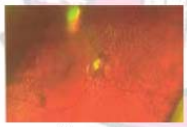
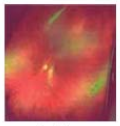
UNINTENDED CONSEQUENCES

- Both true and false positive findings require additional evaluations, leading to additional costs and time away from work
- Increased workload for medical staff related to exams and waivers, loss operational capability due to DNIF
- However, those with pathology may benefit from early diagnosis


Disc Drusen


Drusen


Glaucoma Suspect


Lattice

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RESULTS – COST DATA

- Cost estimates based on RVU's
  - 334 exams per year, at $120 per exam = $41,000 per year
  - Cost of additional follow up for abnormal exams approx $5k/yr
  - Cost of program over the past five years = $339,000
  - Assuming one case of potential retinal pathology per year:
    - Then number needed to screen is 334 (same as exams/yr)
    - Thus cost to have retinal photographs available for the benefit of this aviator is $41k
    - Low cost effectiveness in this specific population

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SCREENING EXAM CRITERIA

- Qualities of a good screening exam include
  - Safe
  - Inexpensive
  - Acceptable to target group
  - Highly reliable and valid
  - Easy to perform and interpret

SCREENING EXAM CRITERIA CONTINUED

- Incidence
  - Events with even a suspected injury are quite low
  - Documented retinal injury almost non-existent
- Prevalence
  - Essentially zero, as any significant retinal damage is symptomatic
  - Aircrew get annual flying exams which screen for retinal pathology (visual acuity and amsler grid)
- Sensitivity/Specificity
  - Not applicable for a baseline (pre-exposure) exam

DISCUSSION

- Retinal photographs are an effective tool for documenting and monitoring retinal pathology
- Use as a baseline or screening tool is less clear
  - + Availability of baseline photos provide comparison for current exam
  - + New findings can be compared to baseline to determine relative age
  - - New findings not necessarily attributed to recent exposure
  - - Clinical correlation required (not diagnostic)
  - - Not all ophthalmologists find baseline photo useful adjunct

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CONCLUSIONS

- Frequency of aircraft cockpit illuminations is increasing
- Incidence of suspected laser eye injuries in aviators remains exceedingly low- virtually no reported cases from multiple military and civilian sources
- Benefit of retinal photographs in evaluating suspected acute laser injuries is debatable
- High rate of asymptomatic findings require further workup
- Test is not cost effective in this population

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RECOMMENDATIONS

- Baseline retinal photographs for all AFSOC flyers are not warranted as a screening exam
- Continued tracking of illuminations/laser injuries essential
- Develop centralized database to capture all laser exposures
- Educate airmen at risk for laser exposure
- Consider providing LEP's to ground troops to protect against friendly fire
- Consider study to determine effectiveness of baseline retinal photographs in other military populations
- Continue to develop broad spectrum LEP's

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- CO-AUTHOR:
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  - Hurlburt Field, FL
- PRECEPTOR:
  - Lee H. Harvis, Col, USAF, MC, SFS
  - Commander, 374th Medical Group
  - Yokota AB, Japan

QUESTIONS?

Visual Performance Enhancement with Macular Pigment in Glare Condition
AFRL 711th Human Performance Wing (HPW)/RHDO

Dr. Leon McLin

PURPOSE: Macular pigment's presence in the fovea is thought to enhance visual performance in glare. This study sought to determine if differences in macular pigment optical density (MPOD) are associated with differences in 3 measures of visual performance under conditions of glare: 1) photostress recovery, 2) disability glare, and 3) visual discomfort. METHODS: Spatial profiles of MPOD were assessed for twenty-six subjects with heterochromatic flicker photometry. Glare was delivered dioptically, in free-view, via two bright white LEDs. For the disability glare and photostress recovery conditions, the visual task consisted of determining the contrast threshold for correct identification of a 1degree Gabor patch's orientation. Visual discomfort was assessed with a visual discomfort rating scale. Pupil diameter was monitored with an IR camera. RESULTS: Low MPODs were associated with higher contrast thresholds even for the control condition without glare. Higher MP values resulted in faster photostress recovery times, lower disability glare contrast thresholds, and lower visual discomfort. Subjects' pupil diameter during glare was significantly correlated with higher visual discomfort ratings. CONCLUSIONS: Macular pigment improves three aspects of visual performance in glare. Unlike previous studies, the present study used free-viewing conditions so effects of iris pigmentation and pupil size could be accounted for. Therefore, the effects described can be extended more confidently to "real-world," practical visual performance benefits.

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Visual Performance Enhancement with Macular Pigment

AFMS Medical Research Symposium
25 Aug 2010

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Co-Authors

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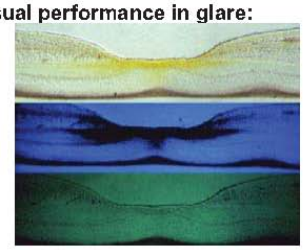
- Maj Brian Foutch - 711 HPW/RHDO, Brooks City-Base
- Peter Smith - TASC Inc.
- Lt Col Michelle Aaron - USAFSAM, Brooks City Base
- Paul Garcia - TASC Inc.

Purpose

- One theory of the function of macular pigment (MP) presence in the fovea is to improve visual performance in glare
- This study sought to determine the effect of MP level on three aspects of visual performance in glare:
  - Photostress recovery
  - Disability glare
  - Visual discomfort

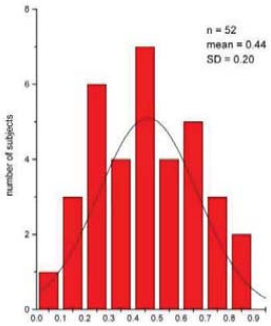


Methods

- Spatial profiles of MP optical density were assessed with heterochromatic flicker photometry for 52 subjects
- Visual performance in glare measured for 26 subjects
  - Disability glare
    - Visual task consisted of correct identification of a 1° Gabor patch's orientation
    - Glare was delivered dioptically, in free-view, via two high-bright white LEDs coupled with diffusers and focusing optics.
  - Photostress recovery – time to recover to see 1° Gabor patch's orientation after a bleaching 5 second central glare exposure
  - Visual discomfort during the glare presentation - assessed with a visual analog discomfort rating scale
- Pupil diameter was monitored with an IR camera, and pupils varied normally

MPODs

- 52 subjects
- Mean = 0.44
- SD = 0.20
- Gaussian-like distribution



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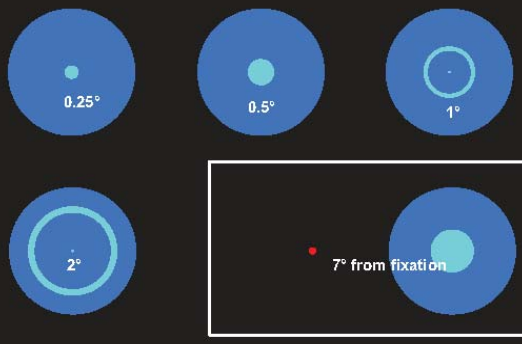
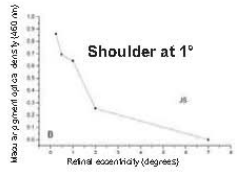
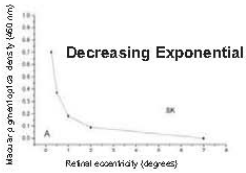
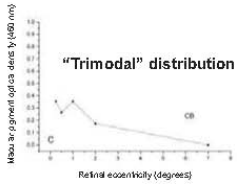
Retinal Locations for MP Measurements



Variations of MPOD spatial profiles







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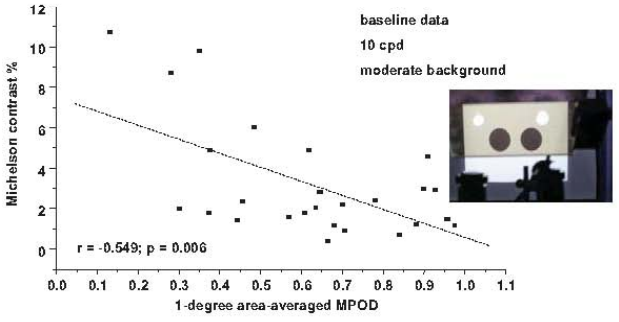
Contrast Thresholds and MPOD

1-degree area-averaged MPOD, Baseline no glare



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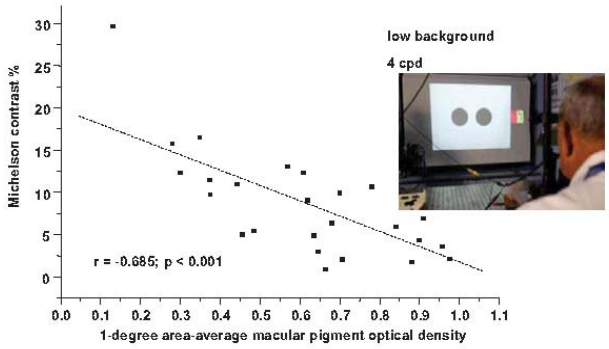
Disability Glare Contrast Thresholds

1-degree area-averaged MPOD, with glare



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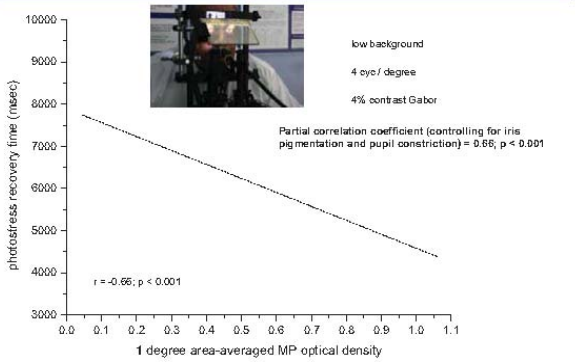
Photostress Recovery



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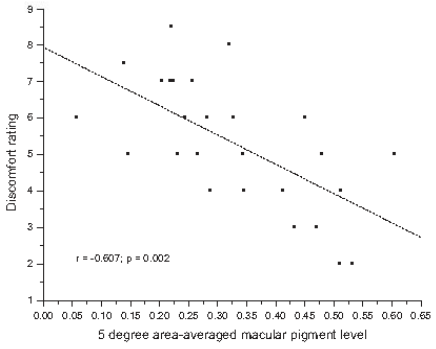
Visual Discomfort Ratings and Central 5° Area-averaged MPOD



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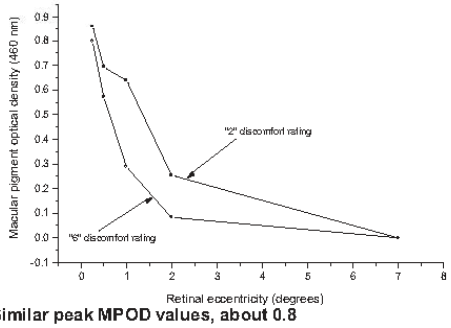
Discomfort ratings for two subjects



- Similar peak MPOD values, about 0.8
- Different central 5° area-averaged MPOD levels, 0.33 and 0.52
- Different discomfort glare ratings, "6" and "2"

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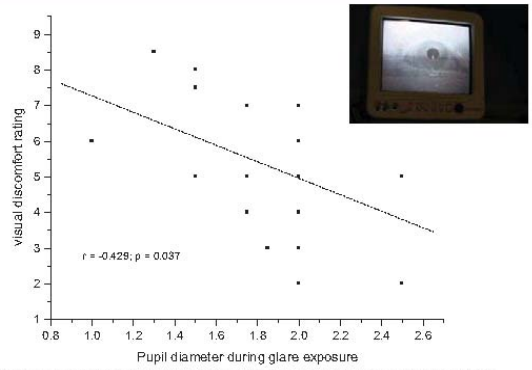
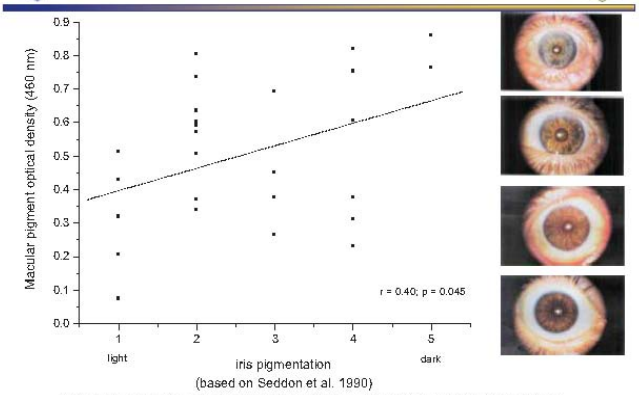
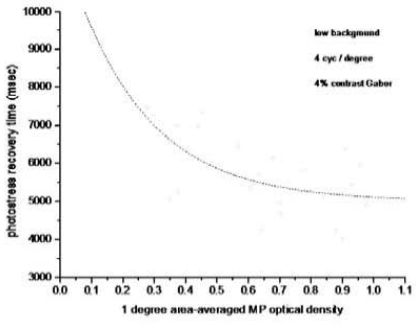
Visual Discomfort Ratings and Pupil Diameter During Glare Exposure



MPOD and Iris Pigmentation



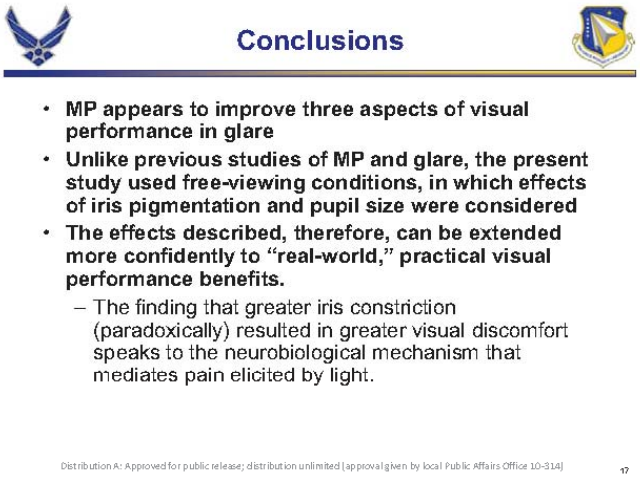
Photostress Recovery and MPOD Fit with Decreasing Exponential Function



The benefit of MPOD for photostess recovery appears to asymptote

Results

- **MP level was found to be significantly correlated with all of the outcome measures.**
  - Higher MP values resulted in:
    - Faster photostress recovery times
    - Lower disability glare contrast thresholds
    - Lower visual discomfort.
  - Smaller pupil diameter during glare presentation was significantly correlated with higher visual discomfort


Conclusions
• MP appears to improve three aspects of visual performance in glare
• Unlike previous studies of MP and glare, the present study used free-viewing conditions, in which effects of iris pigmentation and pupil size were considered
• The effects described, therefore, can be extended more confidently to "real-world," practical visual performance benefits.
– The finding that greater iris constriction (paradoxically) resulted in greater visual discomfort speaks to the neurobiological mechanism that mediates pain elicited by light.
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Identification of Serum Biomarkers of Directed Energy Induced Retinal Injury

United States Army Medical Research Institute of Chemical Defense (USAMRICD)

LTC Deborah Whitmer, 62nd MEDBDE Theater Veterinarian

MILITARY SIGNIFICANCE: Today on the battlefield military personnel are exposed to numerous non-kinetic ballistic risks to their vision such as directed energy (laser) sources and non-penetrating blast effects. Detection of subtle non-penetrating blast and directed energy retinal injuries by retinal specific biomarkers is a desirable field diagnostic capability. OBJECTIVE: Determine if specific retinal biomarkers are measurable in serum after discrete DE retinal injuries occur. METHODS: Laser lesions were created in both eyes of anesthetized non-human primates (NHPs) (n=20 animals). An alpha-2-agonist and non-steroidal anti-inflammatory were administered topically concurrently with a unilateral intravitreal injection (saline or steroid) then evaluated for effects on lesion healing. Serum samples were collected from all NHPs pre and post DE exposure then at regular intervals during the treatment phase and at the terminal end point (minimum of 180 days post retinal injury). The levels of cytokines and chemokines were determined in serum using Millipore's MILLIPLEX™ Non-Human Primate Cytokine kit. The assay coupled with the Luminex xMAP® platform, simultaneously quantified 23 human cytokines and chemokines. RESULTS: Six of 23 assayed serum cytokines/chemokines were detectable at all time points from injury to end point in all NHPs tested. CONCLUSIONS: Preliminary results indicate that specific serum cytokine/chemokines biomarker(s) exist for discrete DE induced retinal injury.

(Not presented)

Proceedings of the 2010 AFMS Medical Research Symposium

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